PREPARATION OF HYARULONIC ACID-BASED HYDROGEL NANOPARTICLES CONTAINING ALPHA ARBUTIN

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MASTER OF SCIENCE

IN

COSMETIC SCIENCE

SCHOOL OF COSMETIC SCIENCE

MAE FAH LUANG UNIVERSITY

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ABSTRACT

The objectives of this study were to synthesize HA- based hydrogel nanoparticles loading alpha arbutin as whitening agent. The hyaluronic acid (HA) and poly (ethylene glycol) diglycidyl ether (PEGDG) as biodegradable and biocompatible materials were used through chemical crosslinking. The hydrogel nanoparticles containing the alpha arbutin as whitening agent were characterized by mean of a scanning electron microscope (SEM), an optical electron microscope. The particles size was studied by SEM and Zetasizer. The HA and PEGDG were confirmed by Fourier transform infrared spectroscopy (FT-IR) and X-ray diffractometry (XRD). The entrapment efficiency and swelling ratio were examined as well. The results showed hydrogel nanoparticles obtained ranged from 800-950 nm at their dried states and above 1496 nm at wetting state. The FT-IR spectrum from stretching vibrations of C-O bond due to the chemically crosslinked hyaluronic acid and PEGDG were observed. From XRD the peaks of hydrogel shift a bit and broader when compare with HA. The Entrapment efficiency is 99.88% and swelling ratio is 8/8 g of hydrogel.

Keywords: HA-based hydrogel nanoparticles/Crosslinking polymerization/ Alpha arbutin

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ABBREVIATIONS AND SYMBOLS

AR	Arbutin
EMC	Extracellular matrix
FT-IR	Fourier transform infrared spectroscopy
g	gram
GAGs	Glucosaminoglycans
HA	Hyaluronic acid
kV	kilovoltage
M	Molar
mA	milliamp
mg	milligram
ml	milliliter
N	Normality
nm	nanometer
PEGDG	Poly(ethylene glycol) diglycidyl ether
R^2	Coefficient of determination
S	standard
SEM	Scanning electron microscope
UV-Vis	ultraviolet visible
XRD	X-ray diffraction
%	percent
λ	Lambda
θ	Theta
∞	Alpha
β	Beta
μ	Micro

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Arbutin is a derivative of hydroquinone that has been found in the leaves of various types of plants, most notably the bearberry plant (*Arctostaphylas uva-ursi*), cranberries and blueberries. It is an inhibitor of melanin formation and use in whitening products. Arbutin is highly hydrophilic and hydroscopic substance but the formidable barrier property of stratum corneum and high hydrophilicity of arbutin make itself difficult to permeate through the skin and reach to target site of action (i.e. melanocytes)[1]. Thus many studies have been used delivery technologies to enhance both stability and skin penetration such as encapsulation arbutin in liposome [2]. The polymeric delivery technology was one of optional resolutions that was applied to improve the stability of arbutin by applying inside of the particles such as a study of chitosan for preparation of arbutin nanoparticles as skin whitening [1].

Development in polymeric delivery for controlled release of therapeutic agents has demonstrated that these systems can not only improve drug stability both in vitro and in vivo by protecting labile drugs from harmful conditions in the body, but also increase residence time at application site and enhance activity duration of short half-life drugs. Therefore, compounds which otherwise would have to be discarded due to stability and bioavailability problems may be rendered useful through a proper choice of polymeric delivery system. Furthermore, polymeric systems can provide predictable and reproducible drug release for extended duration in meeting specific therapeutic requirement, thereby eliminating side effects, frequent dosing, and waste of drugs. One such polymer system for drug delivery applications, which has attached significant recent attention, is based on hydrogels. Hydrogels are hydrophilic network polymers which are glassy in the dehydrated state and swollen in the presence of water to form an elastic gel.

Although hydrogels are of either natural or synthetic origin, they are the covalently crosslinked synthetic hydrogels that have been gaining increasing popularity in various biomedical applications, ranging from soft contact lens to drug delivery systems [3], ever since their introduction more than 20 years ago by Wichterle and Lim [4].

Previous study demonstrated that the hyaluronic acid- based hydrogel nanoparticles were synthesized via a crosslinking reaction between hyaluronic acid (HA) and poly (ethylene glycol) diglycidyl ether (PEGDG) [5]. These components were able to biocompatible with skin and the hyaluronic acid- based hydrogel nanoparticles could penetrate into shallow region of the skin. The study is beneficial to a tropical delivery system due to penetration of the particles are on the surface of the skin. Thus, the aim of this study is to prepare hyaluronic acid- based hydrogel nanoparticles containing alpha-arbutin and to study the physicochemical properties of the obtained arbutin nanoparticles for protecting from degradation.

1.2 Objectives

1.2.1 To prepare of HA –based hydrogel nanoparticles containing alpha-arbutin.

1.2.2 To study physiochemical characterization of HA –based hydrogel nanoparticles containing alpha-arbutin.

1.2.3 To study the swelling and the entrapment efficiency of HA –based hydrogel nanoparticles containing alpha-arbutin.

1.3 Scope of the Study

1.3.1 Preparation of HA –based hydrogel nanoparticles using the inverse suspension (water-in-oil, w/o) polymerization method [5].

1.3.2 Loading alpha-arbutin into HA –based hydrogel nanoparticles.

1.3.3 Physicochemical characterization of HA –based hydrogel nanoparticles containing alpha-arbutin using the following techniques.

A particle size is determined using scanning electron microscope and optical electron microscope [1]. The analysis is performed at a room temperature using dried

samples and dispersing in distilled water samples. In addition, compositions of nanoparticles were observed by using different technique such as FTIR and XRD. Alpha arbutin in nanoparticles is determined by UV-Vis spectroscopy after dispersing in distilled water for 24 hr.

1.3.4 Swelling and entrapment efficiency of HA –based hydrogel nanoparticles containing alpha-arbutin were studied [18].

1.4 Time Line of the Study

Table 1.1 Research activity and timeline

	Activities	Month			
		Apr-14	**May-14**	**Jun-14**	**Jul-14**
1	Synthesize the hydrogel nanoparticles	⟷			
2	Containing alpha arbutin in hydrogel	⟷			
3	Physico chemical characteristic studies	⟷			
4	Alpha arbutin entrapment efficiency study		⟷		
5	Data collection, analysis and conclusion			⟷	⟷

1.5 Expected Outcome

1.5.1 Hyaluronic acid –based hydrogel nanoparticles containing alpha arbutin can be completely synthesized.

1.5.2 Hyaluronic acid and polyethylene glycol diglycidyl ether (PEGDG) can be cross-linked that will not only improved substances deposit into skin but also applied to versatile applications with others substances.

CHAPTER 2

LITERATURE REVIEWS

2.1 Arbutin

Alpha- arbutin is pure, water soluble, biosynthetic active ingredient. Alpha arbutin promotes lightening and even skin tone on all skin types. Alpha- arbutin blocks epidermal melanin biosynthesis by inhibiting enzymatic oxidation of Tyrosinase and Dopa. Structurally, alpha arbutin (IUPAC name = 4-hydroxyphenyl-∞-D-glucopyranoside) (Figure 2.1) is an ∞- glucoside. The ∞- glucosidic bond offers higher stability and efficacy than the β-form in the related beta-arbutin. This leads to skin lightening active that acts faster and more efficiently than existing singles components, minimizes liver spots and reduces the degree of skin tanning after exposure [6].

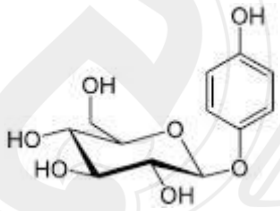

Figure 2.1 Chemical Structure of Alpha arbutin

Due to arbutin is highly hydrophilic and hydroscopic substance but the formidable barrier property of stratum corneum and high hydrophilicity of arbutin make it difficult to permeate through the skin and reach to its site of action (i.e. melanocytes) [1]. In addition, arbutin is a thermodegradable substance [7]. Thus, there were several studies that provided for solutions. One of solutions is formulation of liposome for topical delivery of arbutin. The aims of this study were to encapsulate arbutin (AR) in liposome to enhance

the skin-whitening activity, and to investigate the effect of liposome formulation on the entrapment efficiency (EE %), skin permeation rate and skin deposition. The result of the entrapment efficiency in all liposome formulations vary between 4.35% and 17.63%, and were dependent on the lipid content. The particles sizes of liposomes were in the range of 179.9 ~212.8 nm in all liposome formulations. Although the permeation rate of AR in the liposome formulations decreased compared with AR solution, the deposition amount of AR in the epidermis/dermis layers increased in AR liposomal formulation. These results suggest that liposomal formulation could enhance the skin deposition of hydrophilic skin-whitening agents, thereby enhancing their activities [2].

Application of chitosan is for preparation of arbutin nanoparticles as skin whitening. The arbutin loaded chitosan nanoparticles were prepared by inotropic gelatin technique. These nanoparticles presented high arbutin loading efficiency. The entrapment efficiency increased with increasing arbutin/chitosan weight ratios. This study demonstrated that complexation between chitosan and TPP forms stable cationic nanoparticles for subsequent arbutin loading [1].

Synthesis of arbutin is by two-step reaction from glucose. Arbutin was synthesized from glucose by two-step reaction below: (a) 2, 3, 4, 6-tetra-O-acetyl-∞-D-glucosylchloride or bromide was prepared by glucose and acetyl halide (chloride or bromide). (b) 2, 3, 4, 6-tetra-O-acetyl-∞-D-glucosyl halide (Cl,Br) reacted with hydroquinone, methanol as solvent at PH= 9.5~10. This experiment used toxic substances either halogenating agent or acetyl agent.

Photostability determination of arbutin as a vegetable whitening agent. The target of this research was to evaluate the photostability at various pH values of 0.00012 M aqueous solution of arbutin. The result of this study can be concluded that arbutin is a photostable substance but a thermodegradable substance which can be incorporated in various formulas at room temperature [7].

2.2 Skin and Hydrogel

The skin is the largest organ of the human body, presenting a total area of close to 2 m^2 and acts as a barrier between the organism and the external environment. Important skin functions include protection against UV radiation, physical and chemical damage and microbiological attack, maintenance of the body temperature and sensorial functions such as pain and temperature.

The skin is mainly composed of two layers (epidermis and dermis) besides subcutaneous tissue. It is composed of variety of different cells, being considered more complex than the brain regarding this aspect. The epidermis is composed of several lipids including phospholipids, phosphatidylcholine, cholesterol and triglycerides. The main cell types found in the epidermis are keratinocytes, corneocytes, Langerhans cells, and Merkel cells. The epidermis is divided into several layers and its outermost layer, the stratum corneum, is responsible for the barrier function of the skin due to its lipophilicity and high cohesion between cells. The stratum corneum is composed of keratinized cornecytes embedded in lipid bilayers. Ceramides, cholesterol and free fatty acids comprise its extracellular lipid compartment. The dermis is the layer next to the subcutaneous tissue and it is composed of collagen, elastin, glycosaminoglycans, and fibroblasts. This layer is highly vasicularized besides containing the appendices (sweat glands and pilosebaceous units) and leucocytes, adipocytes and mast cells.

Considering the skin anatomy and physiology, some active substances will not provide the desired activity after their cutaneous administration. Nanotechnology can be used to modify the permeation/penetration by controlling the release of active substances and increasing the period of permanence on the skin besides ensuring a direct contact with the stratum corneum and skin appendices and protecting the active substances against chemical and physical instability. Also, depending on their sizes and structures, some nanostructures can also penetrate across the skin [8].

Recent development in polymeric delivery systems for the controlled release of therapeutic agents has demonstrated that these systems cannot only improve drug stability both in vitro and in vivo by protecting labile drugs from harmful conditions in the body, but also increase residence time at the application site and enhance the activity duration of

short half-life drugs. Therefore, compounds which otherwise would have to be discarded due to stability and bioavailability problems may be rendered useful through a proper choice of polymeric delivery system. Furthermore, polymeric systems can provide predicable and reproducible drug release for extended duration in meeting a specific therapeutic requirement, thereby eliminating side effects, frequent dosing, and waste of drugs [3].

Polymer system for drug delivery applications, which has attracted significant recent attention, is based on hydrogels. Hydrogels are three-dimensional networks of hydrophilic polymer chains that do not dissolve but can swell in water. Because of the hydrophilic properties of polymer chains, they are able to retain a large amount of water within their structures. The high biocompatible hydrogels result from their high water content and soft-surface properties. In addition, hydrogels are versatile materials because they can be tailor-made to process various properties by manipulating the synthetic and processing methods. The physicochemical, mechanical, and biological properties, as well as new functional properties, can be easily modulated. For example, hydrogels can be made respond to environmental stimuli, such as temperature, pH, light, and specific molecules. These interesting properties have made hydrogels useful for various applications ranging from pharmaceutical and biomedical to other industrial applications [9].

2.3 Synthesis of Hydrogel

Several methods have been used to synthesize hydrogels. There are generally two different methods to prepare chemical hydrogels Polymerization of water- soluble monomers in the presence of bi-or multifunctional cross-linking agents result in chemical hydrogels. Typical examples of water-soluble monomers include acrylic acid, acrylamide, hydroxyethyl methacrylate, hydroxypropyl acrylate, and vinylpyrrolidone. Chemical hydrogels can also be prepared by cross-linking water soluble polymers using chemical reactions that involve functional groups of the polymers. Functional groups for cross-linking reactions include vinyl, hydroxyl, amine, and carboxyl groups. Physical hydrogels

are prepared by cross-linking without chemical reactions. Noncovalent bonds can be formed through electrostatic interactions, hydrogen bonding, etc.

2.4 Hyaluronic Acid (HA)

Skin is a large and complex tissue, with a vast range of functions that interfaces with a hostile environment. The mechanisms that underlie the resilience of skin to the harsh outside world, and the extraordinary ability of the skin to also protect underlying tissues are just beginning to be understood. Skin retains a large amount of water, and much of the external trauma to which it is constantly subjected, in addition to the normal process of aging, causes loss of moisture. The key molecule involve in skin moisture is hyaluronan (hyaluronic acid, HA) with its associated water-of-hydration. Understanding the metabolism of HA, its reactions within skin, and the interactions of HA with other skin components will facilitate the ability to modulate skin moisture in a rational manner, different from the empirical attempts that have been utilized up to now.

Recent progress in the details of the metabolism of HA has also clarified the long appreciated observations that chronic inflammation and sun damage caused by ultraviolet light cause premature aging of skin. These processes as well as normal aging utilize similar mechanisms that cause loss of moisture and changes in HA distribution.

In the past several decades, the constituents of skin have become better characterized. The earliest work on skin was devoted predominantly to the cells that make up the layers of skin: epidermis, dermis, and underlying subcutis. Now it is beginning to be appreciated that the materials that lie between cells, the matrix components, have major instructive roles for cellular activities. This extracellular matrix (ECM) endows skin with its hydration properties. The components of ECM, though they appear amorphous by light microscopy, from a highly organized structure of glycosaminoglycans (GAGs), proteoglycans, glycoproteins, peptide growth factors, and structural proteins such as collagen and to a lesser extent, elastin. However, the predominant component of ECM of skin is HA. It is the primordial and the simplest of GAGs, and the first ECM component to be elaborated in the developing embryo. It is the water-of-hydration of HA that forms the blastocyst, the first recognizable structure in embryonic development.

Attempts to enhance the moisture content of skin, in the most elemental terms, require increasing the level and the length of time HA is present in skin, preserving optimal chain length of this sugar polymer, and inducing expression of the profile of HA-binding proteins to decorate the molecule [10].

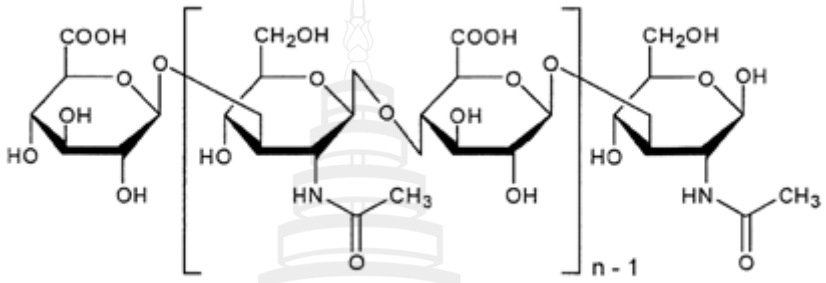

Figure 2.2 Chemical Structure of Hyaluronic acid

2.5 Poly (Ethylene Glycol) Diglycidyl Ether (PEGDG)

Poly (ethylene glycol) diglycidyl ether is widely used as an addition for cross-linking polymers with respect to the biopolymer the biopolymer (influencing the mechanical performance and the stability of the final material). The present invention relates to degradation-stabilized, biocompatible collagen matrices Which are distinguished in particular by the fact they contain soluble collagen and peptide constituents, to processes for the preparation of such collagen matrices, Which processes include in particular chemical crosslinking with an epoxy-functional crosslinking agent, and to the use of the collagen matrices according to the invention as a cosmetic or pharmaceutical agent, in particular for topical use, and also as a wound treatment agent, as an implant or as a haemostatic agent in humans or animals, and as a scaffold for cell population in the biotechnology, basic research and tissue engineering field [11].

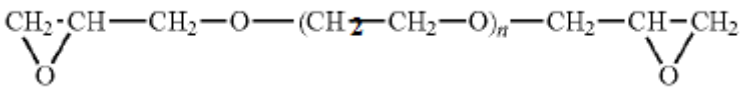

Figure 2.3 Chemical structure of Poly (ethylene glycol) diglycidyl ether [10]

2.6 Characterization of Hydrogel Nanoparticles Using Different Technique

2.6.1 Ultraviolet-Visible spectroscopy

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance. Spectrophotometry is one of the most useful methods of quantitative analysis in various fields such as chemistry, physics, biochemistry, material and chemical engineering and clinical applications [12].

Ultraviolet and visible (UV-Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range. Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels and also is usually applied to molecules or inorganic complexes in solution. The UV-Vis spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The concentration of an analysis in solution can be determined by measuring the absorbance at some wavelength and applying the Beer-Lambert Law. Since, the UV-Vis range spans the range of human visual acuity of approximately 400-750 nm, UV-Vis spectroscopy is useful to characterize the absorption, transmission, and reflectivity of a variety of technologically important materials, such as pigments, coatings and filters. The UV-Vis spectral range is approximately 190 to 900 nm as defined by working range of typical commercial UV-Vis spectrophotometers. The short-wavelength limit for simple UV-Vis spectrometers is the absorption of ultraviolet wavelengths less than 180 nm by atmospheric gases. Purging a spectrometer with nitrogen gas extends this limit to 175 nm.

Working beyond 175 nm requires a vacuum spectrometer and a suitable UV light source. The long-wavelength limit is usually determined by the wavelength response of the detector in the spectrometer.

The light source is usually a deuterium discharge lamp for UV measurements and a tungsten-halogen lamp for visible and NIR (Near Infrared) measurements. The instruments automatically swap lamps when scanning between the UV and visible regions. The wavelengths of these continuous light sources are typically dispersed by a holographic grating in a single or double monochromator or spectrograph. The spectral bandpass is then determined by the monochromator slit width or by the array-element width in array-detector spectrometers. Spectrometer designs and optical components are optimized to reject stray light, which is one of the limiting factors in quantitative absorbance measurements. The detector in single-detector instruments is a photodiode, phototube, or photomultiplier tube (PMT). UV-Vis-NIR spectrometers utilize a combination of a PMT and a Peltier-cooled PbS IR detector. The light beam is redirected automatically to the appropriate detector when scanning between the visible and NIR regions. The diffraction grating and instrument parameters such as slit width can also change. Most commercial UV-Vis absorption spectrometers use one of three overall optical designs: a fixed or scanning spectrometer with a single light beam and sample holder, a scanning spectrometer with dual light beams and dual sample holders for simultaneous measurement of P and Po, or a non-scanning spectrometer with an array detector for simultaneous measurement of multiple wavelengths. In single-beam and dual-beam spectrometers, the light from a lamp is dispersed before reaching the sample cell. In an array-detector instrument, all wavelengths pass through the sample and the dispersing element is between the sample and the array detector [13].

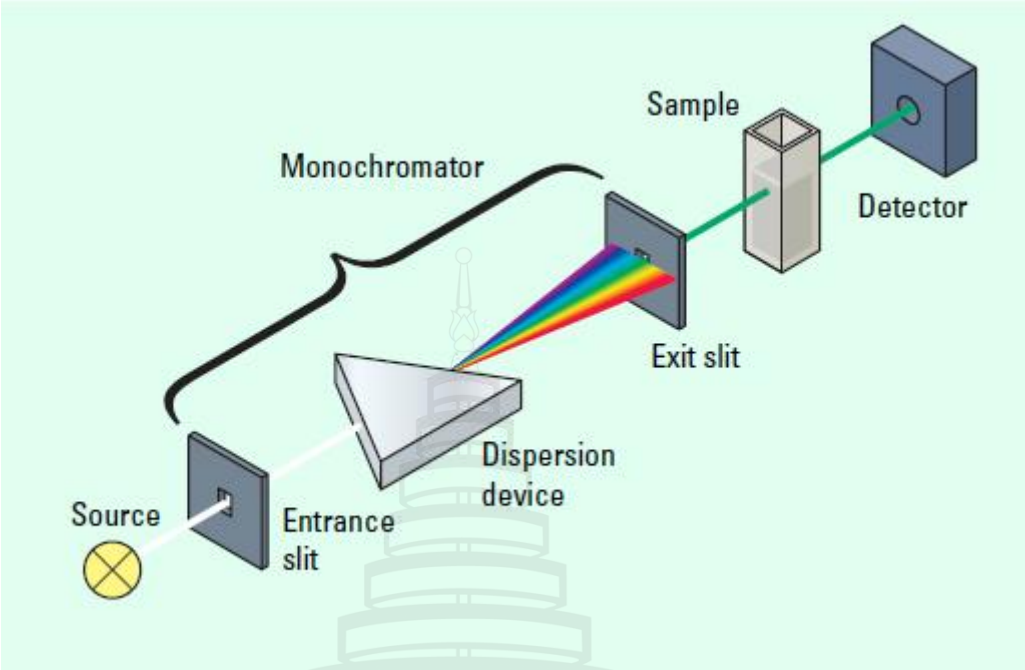

Figure 2.4 Schematic of a conventional spectrophotometer [14]

2.6.2 Fourier transforms infrared spectroscopy (FT-IR)

FT-IR stands for Fourier Transform Infrared, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful to identify unknown material and determine the quality of consistency of sample and amount of components in a mixture.

FT-IR is a method for measuring all of the infrared frequencies simultaneously. The normal instrumental process is as follows (Figure 2.5)

1. The Source: infrared energy is emitted from a glowing black-body source. This beam passes through an aperture which controls the amount of energy presented to the sample (and, ultimately, to the detector).

2. The Interferometer: the beam enters the interferometer where the “spectral encoding” takes place. The resulting interferogram signal then exits the interferometer.

3. The Sample: the beam enters the sample compartment where it is transmitted through or reflected off of the surface of the sample, depending on the type of analysis being accomplished. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.

4. The Detector: the beam finally passes to the detector for final measurement. The detectors used are specially designed to measure the special interferogram signal.

5. The Computer: the measured signal is digitized and sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented to the user for interpretation and any further manipulation.

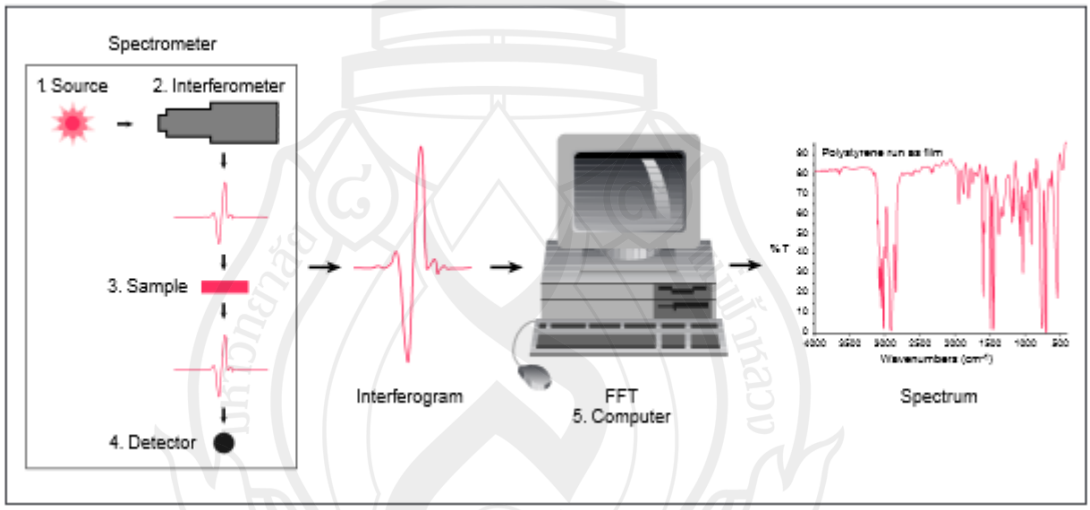

Figure 2.5 The normal process of FT-IR [14]

The signal which exits the interferometers is the result of these two beams interfering with each other. The resulting signal is called an interferogram which has the unique property that every data point (a function of the moving mirror position) which comes from the source. This means that as the interferogram is measured, all frequencies are being simultaneously. Thus, the use of the interferometer result in extreamely fast extremely fast measurements. Finally, a frequency spectrum, a plot of the intensity at each individual frequency is required in order to make identification. The measured interferogram signal can be interpreted via a well-known mathematical technique called

the Fouier transformation. This transformation is performed by the computer which then presents the user with the desired spectral information foe analysis [16].

2.6.3 X-Ray diffraction (XRD)

X-ray diffraction is an important technique in the field of materials characterization to obtain structural information on an atomic scale from both crystalline and non-crystalline (amorphous) materials. XRD technique requires an X-ray source (X-ray tube) which is enabled to probe crystalline structure at the atomic level, the sample which is under investigation and a detector to pick up the diffracted X-rays (Figure 2.6)

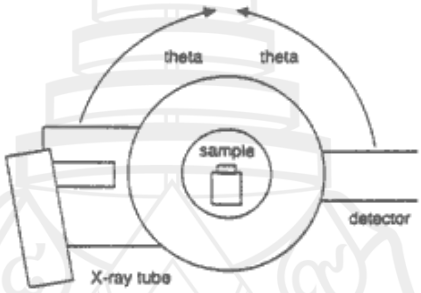

Figure 2.6 Simplified sketch of one possible configuration of the X-ray source (X-ray tube), the X-ray detector, and the sample during an X-ray scan. In this configuration, the X-ray tube and the detector both move through the angle theta (θ), and the sample remains stationary.

The three-dimensional structure of nonamorphous materials, such as minerals, is defined by regular, repeating planes of atoms that form a crystal lattice. When a focused X-ray beam interacts with these planes of atoms, part of the beam is transmitted, part is absorbed by the sample, part is refracted and scattered, and part is diffracted. Diffraction of an X-ray beam by a crystalline solid is analogous to diffraction of light by droplets of water, producing the familiar rainbow. X-rays are diffracted by each mineral differently, depending on what atoms make up the crystal lattice and how these atoms are arranged.

In X-ray powder diffractometry, X-rays are generated within a sealed tube that is under vacuum. A current is applied that heats a filament within the tube; the higher the current the greater the number of electrons emitted from the filament. This generation of

electrons is analogous to the production of electrons in a television picture tube. A high voltage, typically 15-60 kilovolts, is applied within the tube. This high voltage accelerates the electrons, which then hit a target, commonly made of copper. When these electrons hit the target, X-rays are produced. The wavelength of these X-rays is characteristic of that target. These X-rays are collimated and directed onto the sample, which has been ground to a fine powder (typically to produce particle sizes of less than 10 microns). A detector detects the X-ray signal; the signal is then processed either by a microprocessor or electronically, converting the signal to a count rate. Changing the angle between the X-ray source, the sample, and the detector at a controlled rate between preset limits is an X-ray scan

When an X-ray beam hits a sample and is diffracted, we can measure the distances between the planes of the atoms that constitute the sample by applying Bragg's Law. Bragg's Law is:

$$n\lambda = 2d\sin\theta$$

where the integer n is the order of the diffracted beam, λ is the wavelength of the incident X-ray beam, d is the distance between adjacent planes of atoms (the d-spacings), and θ is the angle of incidence of the X-ray beam. Since we know and we can measure, we can calculate the d-spacings.

The geometry of an XRD unit is designed to accommodate this measurement. The characteristic set of d-spacing generated in a typical X-ray scan provides a unique “fingerprint” of the mineral or minerals present in the sample. When properly interpreted, by comparison with standard reference patterns and measurements, this “fingerprint” allows for identification of the material [17].

2.6.4 Scanning electron microscope (SEM)

The SEM [18] is a microscope that uses electrons instead of light to form an image. Since their development in the early 1950’s, scanning electron microscopes have developed new areas of study in the medical and physical science communities. The SEM has allowed researchers to examine a much bigger variety of specimens.

The scanning electron microscope has many advantages over traditional microscopes. The SEM has a large depth of field, which allows more of a specimen to be in focus at one time. The SEM also has much higher resolution, so closely spaced

specimens can be magnified at much higher levels. Because the SEM uses electromagnets rather than lenses, the researcher has much more control in the degree of magnification. All of these advantages, as well as the actual strikingly clear images, make the scanning electron microscope one of the most useful instruments in research today.

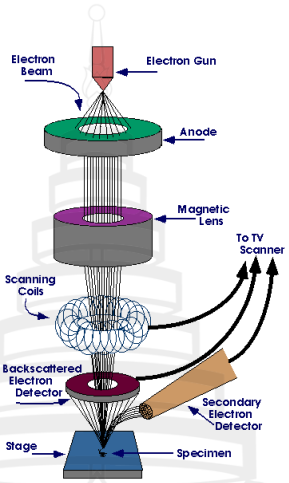

Figure 2.7 Schematic drawing of the scanning electron microscope

The SEM is an instrument that produces a largely magnified image by using electrons instead of light to form an image. A beam of electrons is produced at the top of the microscope by an electron gun. The electron beam follows a vertical path through the microscope, which is held within a vacuum. The beam travels through electromagnetic fields and lenses, which focus the beam down toward the sample. Once the beam hits the sample, electrons and X-rays are ejected from the sample.

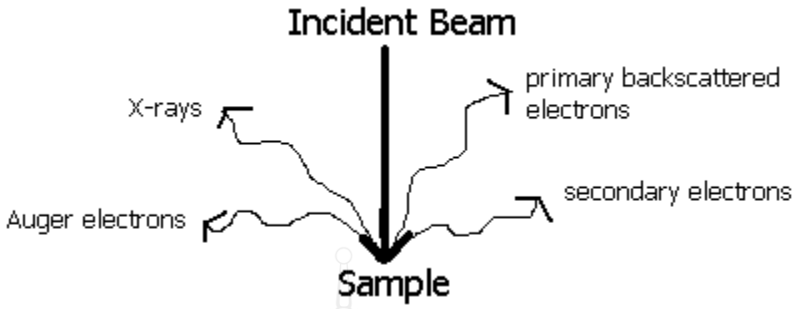

Figure 2.8 Detectors collect these X-rays, backscattered electrons, and secondary electrons and convert them into a signal that is sent to a screen similar to a television screen.

Due to the SEM utilizes vacuum conditions and uses electrons to form an image, special preparations must be done to the sample. All water must be removed from the samples because the water would vaporize in the vacuum. All metals are conductive and require no preparation before being used. All non-metals need to be made conductive by covering the sample with a thin layer of conductive material. This is done by using a device called a "sputter coater."

The sputter coater uses an electric field and argon gas. The sample is placed in a small chamber that is at a vacuum. Argon gas and an electric field cause an electron to be removed from the argon, making the atoms positively charged. The argon ions then become attracted to a negatively charged gold foil. The argon ions knock gold atoms from the surface of the gold foil. These gold atoms fall and settle onto the surface of the sample producing a thin gold coating [18].

2.6.5 Zetasizer Nano ZS

The Zetasizer Nano ZS [19] is a high performance two angle particle and molecular size analyzer for the enhanced detection of aggregates and measurement of small or dilute samples, and samples at very low or high concentration using dynamic light scattering with ‘NIBS’ optics. The ZSP also incorporates a zeta potential analyzer that uses electrophoretic light scattering for particles, molecules and surfaces, and a molecular weight analyzer using static light scattering. Using Non-Invasive Backscatter optics (NIBS) it has significantly better performance than systems using 90 degree

scattering optics. In addition, a microrheology option is available for measuring sample viscosity and viscoelastic properties, as well as a Protein Measurement option for protein mobility measurements.The flow mode option enables the system to be connected to an SEC or an FFF system to use as a detector for the size of proteins or nanoparticles. A choice of cuvettes are available, from disposable single-use to specific cells for viscous or high concentration samples or measuring the zeta potential of surfaces.

The Zetasizer Nano ZS incorporates three techniques in a single compact unit, and has a range of options and accessories to optimize and simplify the measurement of different sample types.

1. Dynamic Light Scattering is used to measure particle and molecule size. This technique measures the diffusion of particles moving under Brownian motion, and converts this to size and a size distribution using the Stokes-Einstein relationship. Non-Invasive Back Scatter technology (NIBS) is incorporated to give the highest sensitivity simultaneously with the highest size and concentration range. Measurement of size as a function of concentration enables the calculation of k_D, the DLS interaction parameter. The Microrheology option uses the DLS measurement of tracer particles to probe the structure of dilute polymer and protein solutions.

2. Laser Doppler Micro-electrophoresis is used to measure zeta potential. An electric field is applied to a solution of molecules or a dispersion of particles, which then move with a velocity related to their zeta potential. This velocity is measured using a patented laser interferometric technique called M3-PALS (Phase analysis Light Scattering). This enables the calculation of electrophoretic mobility and from this the zeta potential and zeta potential distribution. A surface zeta potential accessory uses tracer particles to measure electro-osmosis close to a sample surface to calculate the zeta potential of the surface.

3. Static Light Scattering is used to determine the molecular weight of proteins and polymers. In this technique, the scattering intensity of a number of concentrations of the sample is measured, and used to construct a Debye plot. From this the average molecular weight and second virial coefficient can be calculated, which gives a measure of molecule solubility. This technique is very demanding on the sensitivity and stability of the whole system, and requires that every element of the design is optimized to ensure accuracy and repeatability

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Chemicals

Hyaluronic acid (HA,M_n ~1500000)	(M.C.Biotec.Inc, China)
Poly (ethylene glycol) diglycidyl ether	(Sigma-Aldrich, USA)
Dodecane	(Sigma-Aldrich,USA)
NaOH	(Sigma-Aldrich,USA)
Acetone	(Sigma-Aldrich,USA)
Acetic acid	(Sigma-Aldrich,USA)
PEG 30(Arlacel P 135)	(Croda International)
Alpha-arbutin	(Thornhill advances Research Inc, CA)

3.2 Equipments

2- Digit digital balance	(Adventurer/ARC 120, USA)
4- Digit digital balance	(Denver/TB214, USA)
Hotplate stirrer	(Schott/GmbH, Germany)
Homogenizer	(IKA® /T25, Germany)
Round bottle flask	(Duran, Germany)
Beaker	(Duran, Germany)
Cylinder	(Duran, Germany)
Centrifuge	(Hettich/Mikro 22 R, USA)
Filter paper No.1	(Whatman, Germany)
UV-Vis Spectrophotometer	(Biochroms/Libra S22, UK)
FT-IR Spectrophotometer	(Perkin Elmer/ FTIR Spectrum GX, USA)
X-Ray diffractometer	(PANalytical/ X'Pert Pro MPD, Netherland)

SEM	(LEO 1450 VP)
Zetasizer	(Malvern/Zetasizer Nano ZS9, England)
Optical microscope	(Nikon/Nikon eclipse e 200, Japan)

3.3 Methodology

3.3.1 Hyaluronic acid (HA) based hydrogel nanoparticles praparation

Nanoparticles were prepared by the inverse emulsion polymerization method. According to the previous report [5] with modification hyaluronic acid (HA) (0.34 g) and poly (ethylene glycol) diglycidyl ether (PEGDG) (0.79 g) were dissolved in 11.25 ml of 0.1 N sodium hydroxide aqueous solution. While stirring, the aqueous solution was added slowly to 27.16 ml of dodecane. Emulsion was homogenized for 13 minutes. For an initial crosslinking reaction, the emulsion was transferred into a round bottom flask. It was heated up to 60 ˚C while stirring for 1 hour. Then, the emulsion was cooled at room temperature, and 0.065 ml of acetic acid was added to the emulsion to neutralize the aqueous phase. For a post-crosslinking reaction was left at room temperature for 2 days. After synthesis, the emulsion was transferred slowly to acetone and dried in hot air oven at 90 ˚C for 24 hours.

3.3.2 Alpha arbutin loading

Alpha- arbutin was loaded in the hydrogel nanoparticles using the postpolymerization method. About 1.4 mg of the alpha- arbutin powder was dispersed in the aqueous phase before emulsification. Then follow the steps as HA based hydrogel nanoparticles preparation.

3.3.3 Characterization of the synthesized hyarulonic acid–based hydrogel nanoparticles containing alpha arbutin

3.3.3.1 Scanning electron microscope (SEM).

The estimated size and morphology of HA-based hydrogel nanoparticles containing alpha arbutin were observed by using Scanning electron microscope (LEO 1450 VP). The samples were put on the aluminum tapes which were glued on the roller plate and covered by spraying golden. The samples were observed at 1000X and size

distribution was measured 428 times. The size distribution was presented by using Microsoft Excel program.

3.3.3.2 Zetaseizer

The Zetasizer was obtained by using zeta sizer (Malvern/Zetasizer Nano ZS9, England). The sample 0.02 g was dispersed in DI water 20 ml. The sample was measured size after leaving 24 hours.

3.3.3.3 Fourier transformed infrared spectroscopy (FT-IR)

The FT-IR spectra were obtained by using FT-IR spectrophotometer (Perkin Elmer/ FTIR Spectrum GX). The samples were mixed uniformly with potassium bromide (KBr) at 1:100 (sample: KBr) ratio respectively. The KBr discs were prepared by compressing the mixed powders in a hydraulic press. The disc was scanned in the range of 4000- 400 cm^{-1} to obtain spectra.

3.3.3.4 X-Ray diffraction

XRD patterns were obtained at room temperature using XRD diffractometer (PANalytical/ X'Pert Pro MPD), with Cu Kα target tube, NaI detector, variable slits, a 0.050 step size, operated at a voltage of 30 kV, 15 mA current, at 2 theta/ min scanning angles ranged from 0 to 80^{o}.

3.3.3.5 UV-vis spectroscopy

The alpha arbutin in hydrogel nanoparticles which dispersed in distilled water was detected at 288 nm.

3.3.4 Entrapment efficiency by indirect method [21]

Aqueous phase after crosslinking polymerization and acetone for washing wastes were kept to detect the alpha arbutin that was unloaded. Alpha arbutin was detected at 288 nm by UV-Vis spectroscopy. Entrapment efficiency can be calculated with the following equation:

Entrapment efficiency (%) = W_A-W_a/W_A*100

W_A is the total amount of alpha arbutin and

W_a is the unloaded amount of alpha arbutin

3.3.5 Swelling studies [21]

Swelling behavior (water uptake kinetic and equilibrium swelling) of the hydrogels was evaluated in distilled water by means of gravimetric measurements using an analytical balance (Denver/TB214, USA). In particular, samples were immersed into distilled water (3 ml distilled water/ 4 mg of sample) and weight after being kept at 37 ˚C for 24 hours. The swelling ratio was calculated as follows:

Swelling ratio = $Ws_{(g)}$-$Wd_{(g)}$/$Wd_{(g)}$*100

Ws is the swollen sample weight

Wd is the dried initial sample weight.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Synthesis of HA-Based Nanogels Loading Alpha Arbutin

The purpose of this study is to develop novel hydrogel nanoparticles- loaded alpha arbutin as whitening agent. It was successfully prepared by the inverse emulsion polymerization method. In this study, hyaluronic acid (HA) and poly (ethylene glycol) diglycidyl ether (PEGDG) were chosen as components of hydrogel nanoparticles. Both are biocompatible and highly water soluble, and HA is a natural polysaccharide which take up tremendous amount of water. Therefore, it is expected that the combination of the two chemical compounds might produce suitable hydrogel nanoparticles as delivery carriers. Moreover, HA is one of the NMF (natural moisturizing factor) that it might help boost up moisturizer on the skin during the releasing of alpha arbutin.

The inverse emulsion polymerization technique produced the hyaluronic acid-based hydrogel nanoparticles. An aqueous phase was prepared by solubilizing the two polymers (hyaluronic acid and poly [ethylene glycol] diglycidyl ether) in sodium hydroxide aqueous solution. The alpha arbutin, white crystalline powder, was dissolved in the aqueous phase. As an oil phase, dodecane containing Arlacel P 135, a surfactant, were solubilized. The aqueous phase was dropped into oil phase by slowly while stirring. The emulsion was homogenized at 6000 rpm for 13 minutes. The homogenizer should be held tightly and put deep enough to remain the speed and mix well otherwise the emulsion would separate obviously. Emulsion in a round bottom flask was left for two days before harvesting the hydrogel nanoparticles. After synthesis, the hydrogel nanoparticles fell down the bottom of flask. The oil phase that floated on the upper layer of emulsion was separated neatly. The acetone was used in washing step by repeating three times. The resulting hyaluronic acid-based hydrogel nanoparticles loaded alpha arbutin were

collected and dried at 90˚C for 24 hours. As shown in Figure 4.1, the hydrogel nanoparticles appeared white and small plates.

Figure 4.1 Image of HA-based hydrogel nanoparticles loaded alpha arbutin

4.2 Characterization of HA-Based Nanogels Loading Alpha Arbutin

4.2.1 Scanning electron microscope (SEM)

Scanning electron microscopes image at 1000x (Figure 4.2) shows a cluster of the HA- based hydrogel nanoparticles loaded alpha-arbutin. The surface of each particle linked to other surfaces' particles as jelly or glue. This implied that the nanogels were expanded isotropically due to the uptake of tremendous of water. Meanwhile, the shape of a few particles deviated from spherical shape, indicating that these nanogels were aggregated.

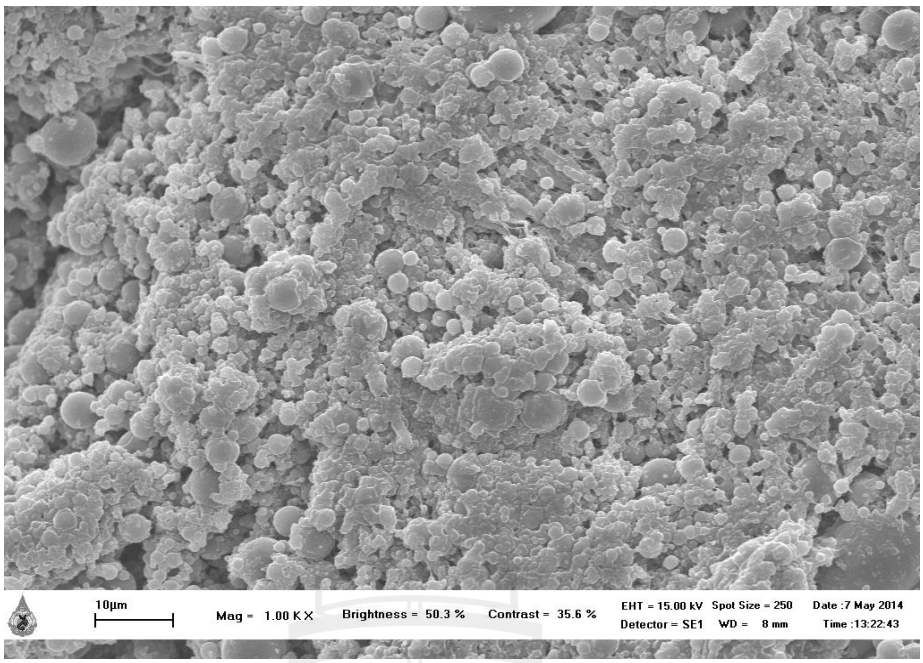

Figure 4.2 SEM of the alpha arbutin HA- based hydrogel nanoparticles at dried state

The particles' size was studied by measuring particles in the SEM image and then arrange in ranging of nanometer (Figure 4.3). Most of nanoparticles obtained ranged from 800-950 nm with a quite broad size distribution at dried state. In addition, Figure 4.4 is an optical microscope image of the alpha arbutin HA based nanogels dispersed in water for 24 hours. The alpha arbutin nanogels were swelled and most size is less than 0.05 mm. These results show HA- based nanogel loading alpha arbutin significantly expand their volumes after uptake of water.

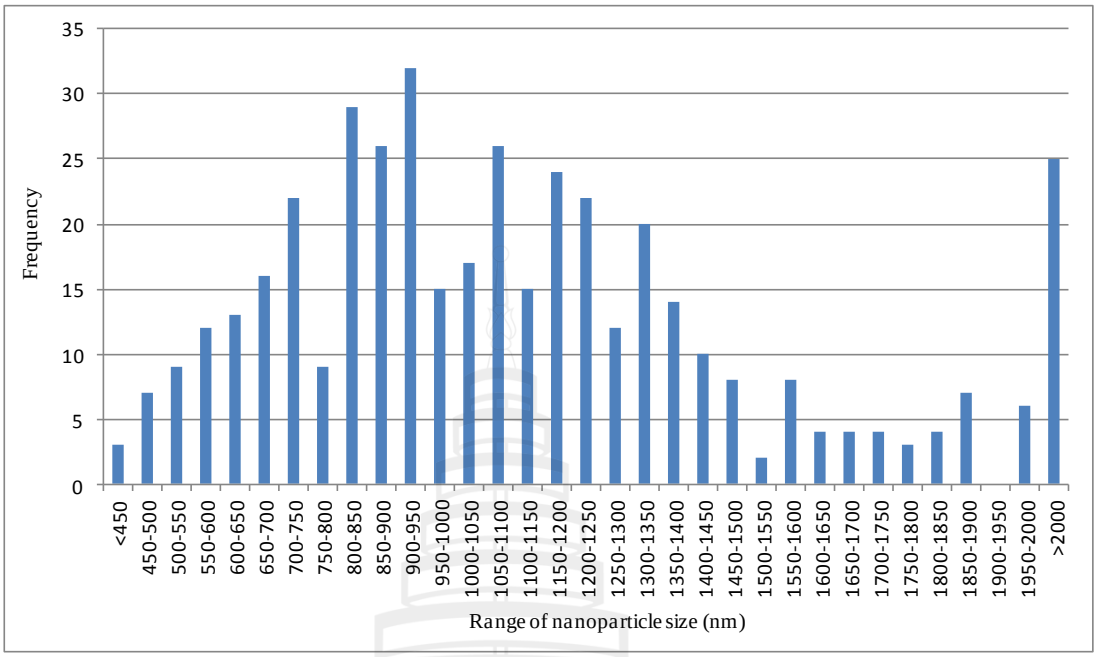

Figure 4.3 Size distribution of HA-based hydrogel nanoparticles loaded alpha arbutin at dried state.

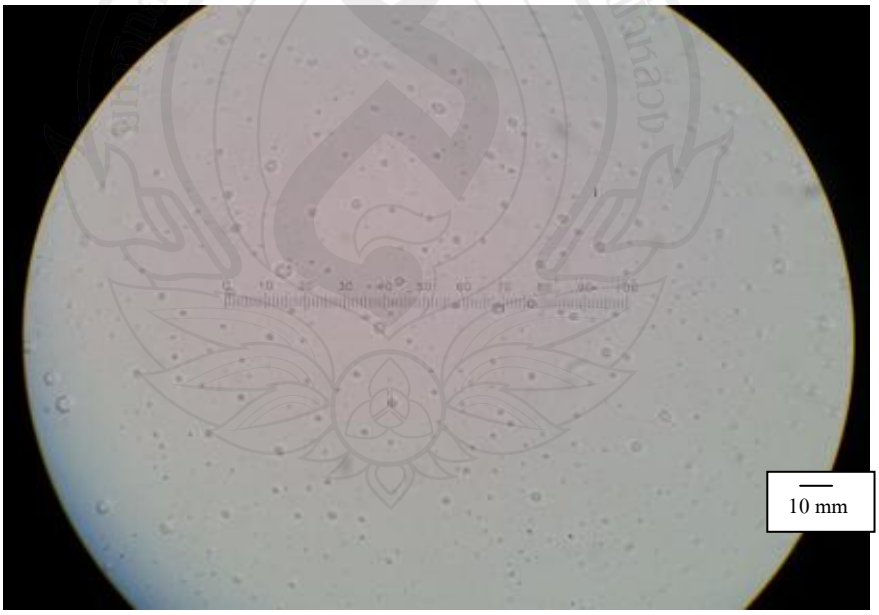

Figure 4.4 An optical microscopy image of the alpha arbutin HA- based hydrogel nanoparticles after being dispersed in water for 24 hours.

4.2.2 Zetasizer

The particles' sizes at wetting state were measured by intensity by three times (Figure 4.5, 4.6, and 4.7) after swelling in distilled water for 24 hours. The average intensities (89.87 %) points that most hyaluronic acid- based hydrogel nanoparticles contained alpha arbutins' size were 1496 nm. On the other hand the rest of intensities (10.13%) showed at 5408 nm. The broad distribution of particles' size might result from the speed and time of homogenization, were not enough to obtain small and narrow size distribution. Moreover, vacuum drying may also help to evaporate water from hydrogel nanoparticles that provide smaller size.

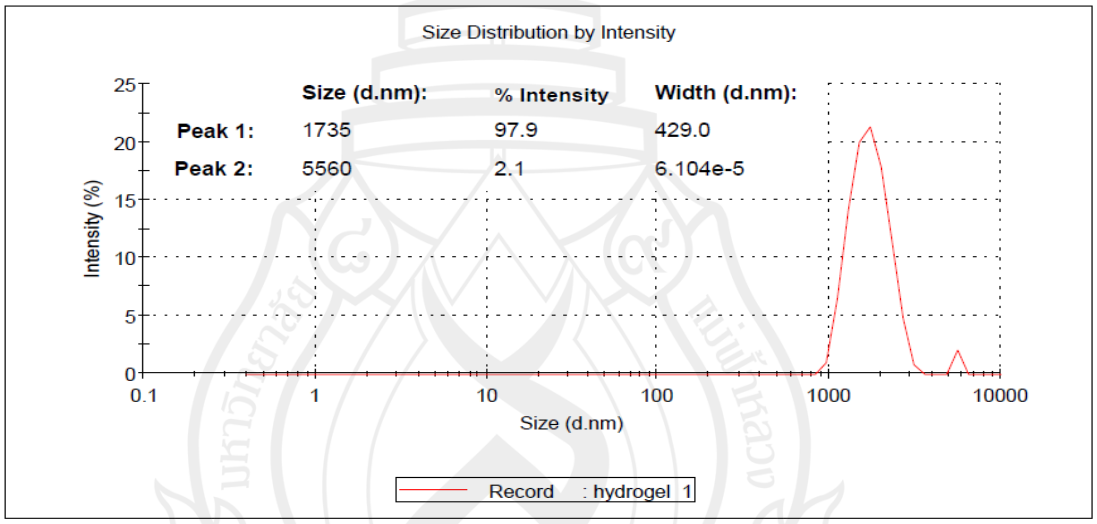

Figure 4.5 Hydrogel 1: Zetasizer image of the HA- based hydrogel nanoparticles loaded alpha arbutin after being dispersed in water for 24 hours.

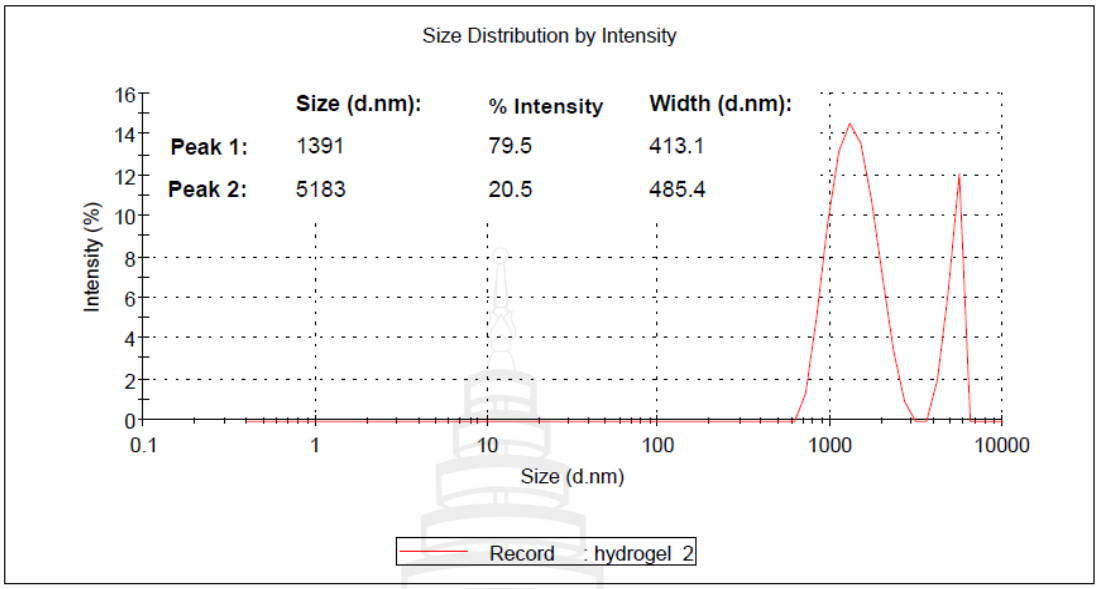

Figure 4.6 Hydrogel 2: Zetasizer image of the HA- based hydrogel nanoparticles loaded alpha arbutin after being dispersed in water for 24 hours.

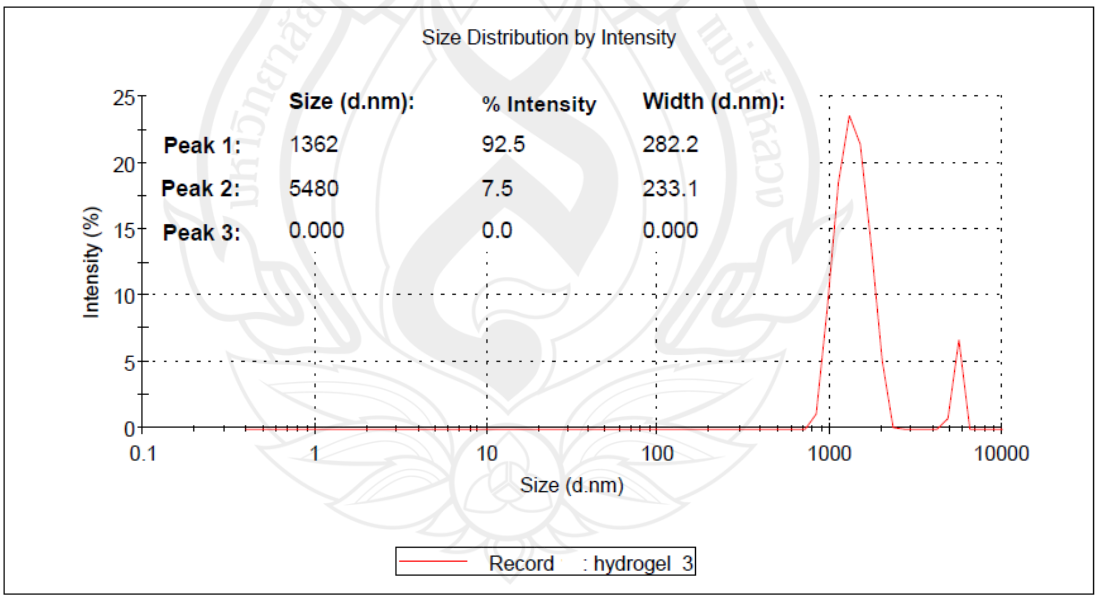

Figure 4.7 Hydrogel 3: Zetasizer image of the HA- based hydrogel nanoparticles loaded alpha arbutin after being dispersed in water for 24 hours.

4.2.3 Fourier transformed infrared spectroscopy (FT-IR)

The FT-IR spectrum of HA as shown in Figure 4.8 shows the strong band at about 3399 cm^{-1} is rather broad and can be assigned to hydrogen-bonded O-H and N-H stretching vibrations. A group of overlapping bands of moderate intensity is observed around 2891 cm^{-1} which are due to the C-H stretching vibrations. The bands at 1621 and 1412 cm^{-1} can be attributed to the asymmetric (C=O) and symmetric (C-O) stretching modes of the planar carboxyl groups in the hyaluronic acid. The three signals centered at 1152, 1056, and 1039 cm^{-1} are assigned to C-O-C (O-bridge), C-O (exocyclic), and C- H group, respectively. The band at 947 cm^{-1} can be assigned to asymmetrical out-of-phase ring vibration [20].

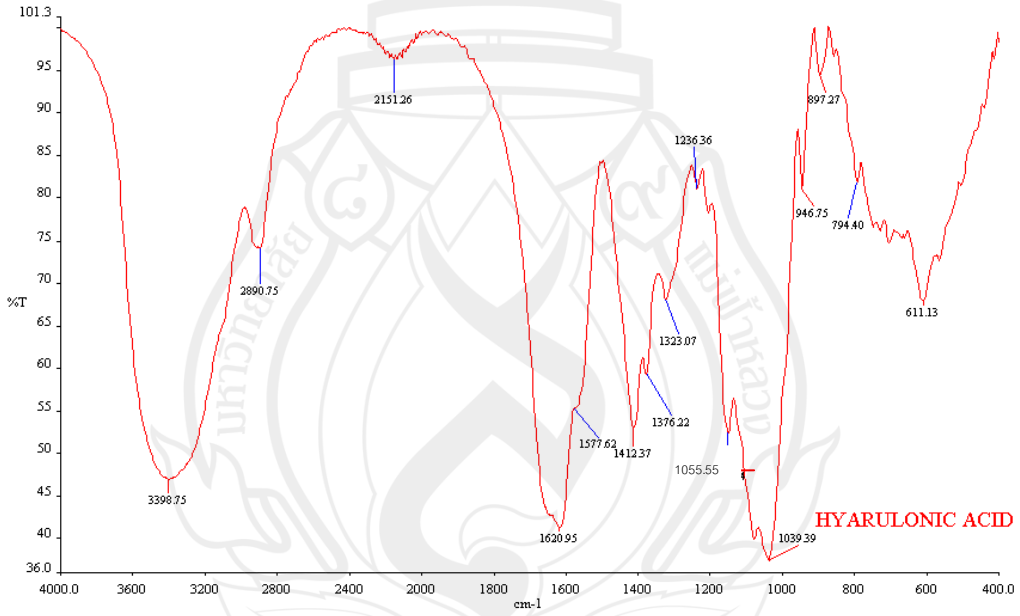

Figure 4.8 FT-IR spectrum of Hyarulonic acid (HA)

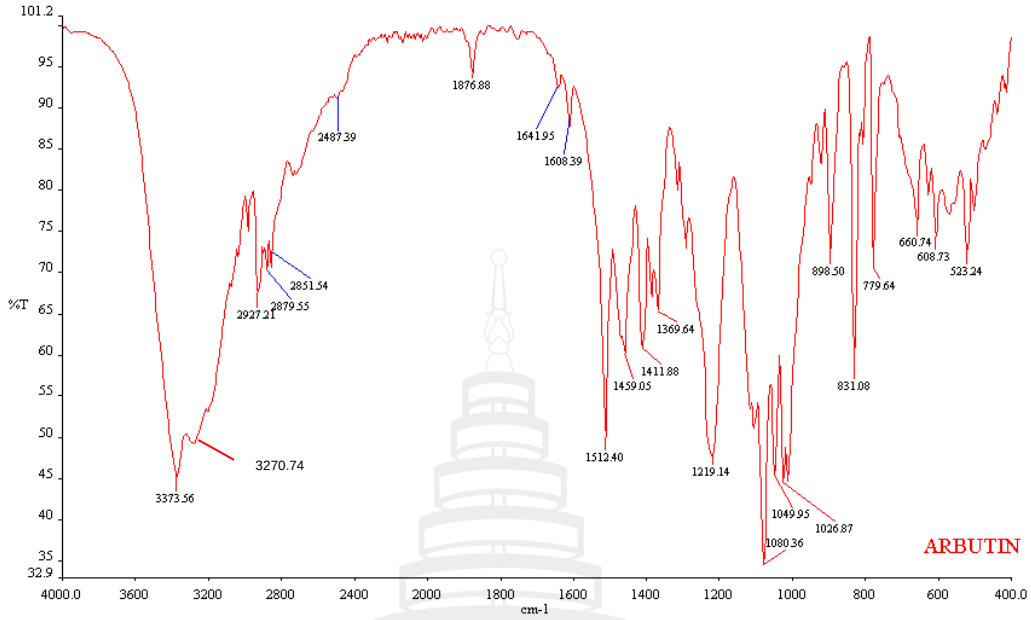

Figure 4.9 FT-IR spectrum of alpha arbutin

FT-IR spectrum of alpha arbutin in Figure 4.9 shows O-H stretching vibration and =CH bonding at 3374 cm^{-1} and 3271 cm^{-1}, respectively. The multiple spectrums range in between 2600- 2900 cm^{-1} can be attributed to –CH stretching frequency. The sharp spectrum of C=C in aromatic shows at 1512 cm^{-1}. The CH_3 bending and CO stretching frequencies were observed between 1300-1400 cm^{-1} and 1000-1200 cm^{-1}, respectively.

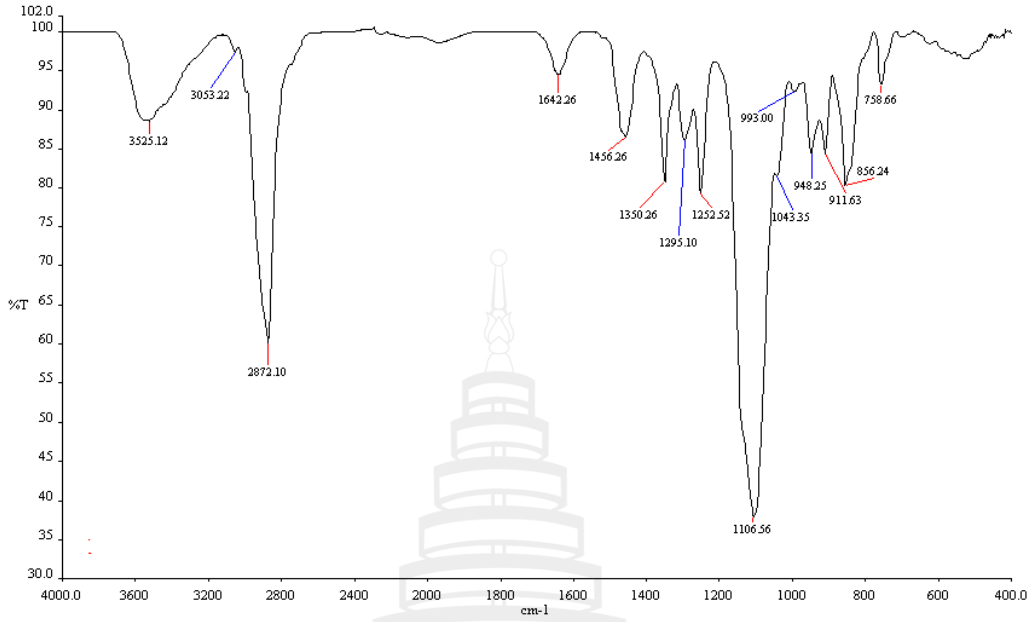

Figure 4.10 FT-IR spectrum of poly ethylene glycol diglycidyl ether (PEGDG)

In Figure 4.10 shows FT-IR spectrum of PEGDG that the peaks at 2872 cm^{-1} and 1107 cm^{-1} were observed prominently due to –CH stretching multiples and C-O-C stretching.

FT-IR spectrum of HA-based hydrogel nanoparticles loaded alpha arbutin is shown in Figure 4.11. The overlapping spectrum at 3401 nm^{-1} can be observed due to –OH groups and -NH of hyaluronic acid remained after crosslinking polymerization and the CH stretching vibration spectrum of hyaluronic acid and PEGDG shifted to 2851 and 2924 nm^{-1}, respectively. The spectrum of C=N bonding at 2308 cm^{-1} is able to confirm hyaluronic acid component. The peak at 1085 cm^{-1}, 1043 cm^{-1} can be attributed to stretching vibrations of C-O stretching due to crosslinking polymerization at ether group of PEGDG and carboxyl group of HA as shown in Figure 4.12 . The FT-IR spectroscopic study confirmed that the hydrogel composed of reaction between PEGDG and hyaluronic acid.

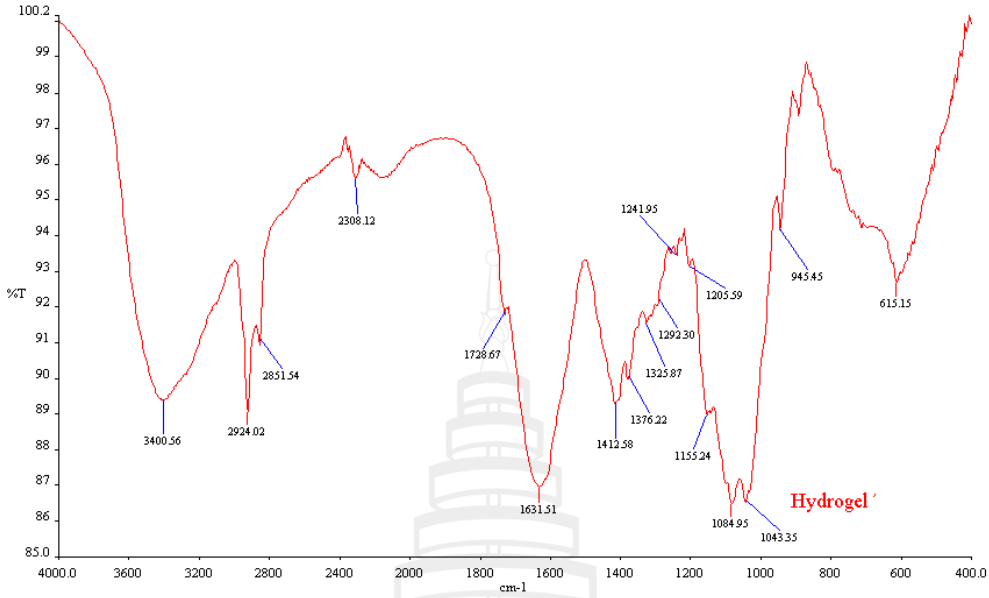

Figure 4.11 FT-IR spectrum of alpha arbutin loading hydrogel nanoparticles

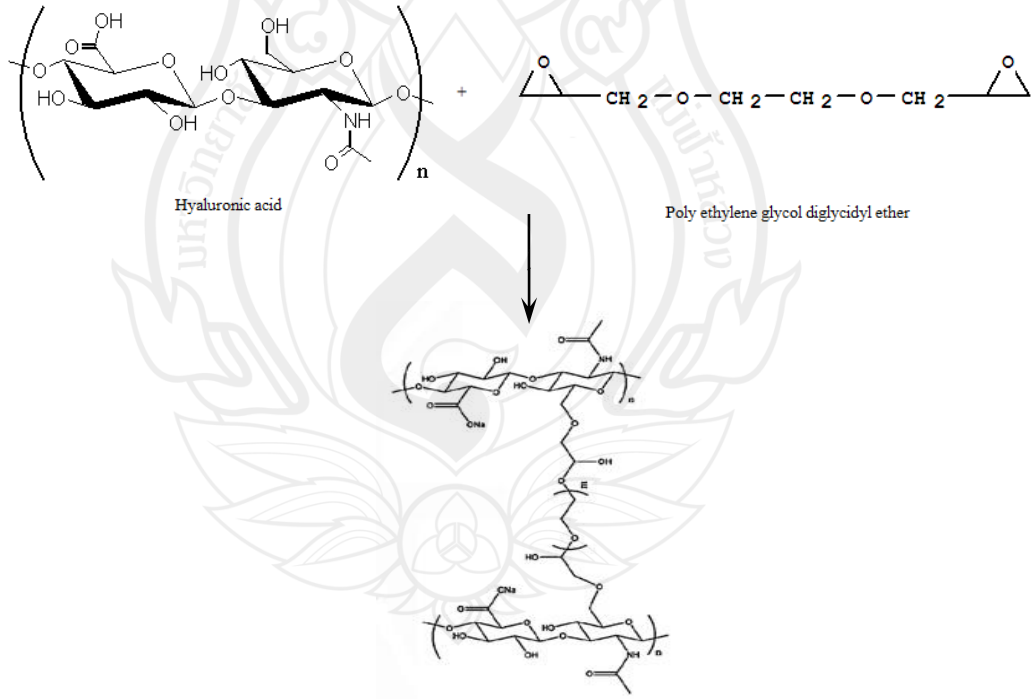

Figure 4.12 Synthesis of HA-based nanogel

4.2.4 X- ray diffractometry (XRD)

The XRD clearly indicates that the HA-based hydrogel nanoparticles formed by reaction between HA and PEGDG as shown in Figure 4.13 and 4.14. The confirmation was observed from the characteristic peaks of HA and hydrogel nanoparticles that both peaks are shown the same peak at nearby previous positions but the intensity were changed. Moreover, the broad peak changed to broader peak that because of shifting to lower intensity because of crosslinking polymerization. The unit cell parameter was estimated by using 2 theta at room temperature.

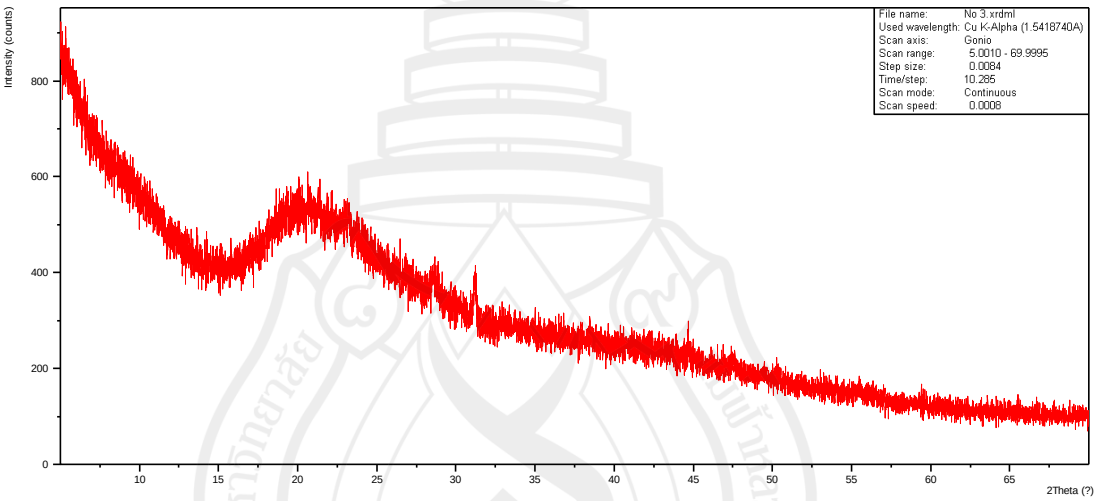

Figure 4.13 X-ray diffractogram of hyaluronic acid (HA) at room temperature.

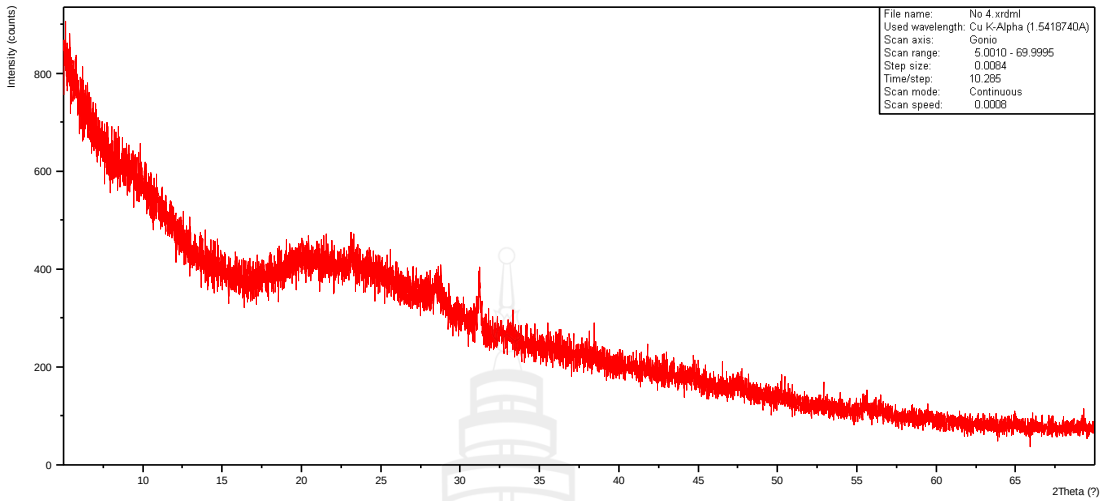

Figure 4.14 X-ray diffractogram of HA-based hydrogel nanoparticles containing alpha arbutin at room temperature

4.3 Entrapment Efficiency by Indirect Method [22]

4.3.1 System linearity

The Alpha arbutin solution 0.02 mg/ml was scanned at between 200-700 nm by UV-vis spectrophotometer. The highest absorbance of alpha arbutin was happened at 288 nm.

The standard blank, 0.2 (S_1), 0.4 (S_2), 0.6 (S_3) and 0.8 (S_4) µg/ml (prepared as described in appendix) in cuvettes were put for four absorbances at each point construct the calibration curve between absorbance and concentrations. The regression line by method of least squares was established. The coefficient of determination (R^2) of the alpha arbutin from a fitted linear regression was 0.9968 as shown in Figure 4.15.

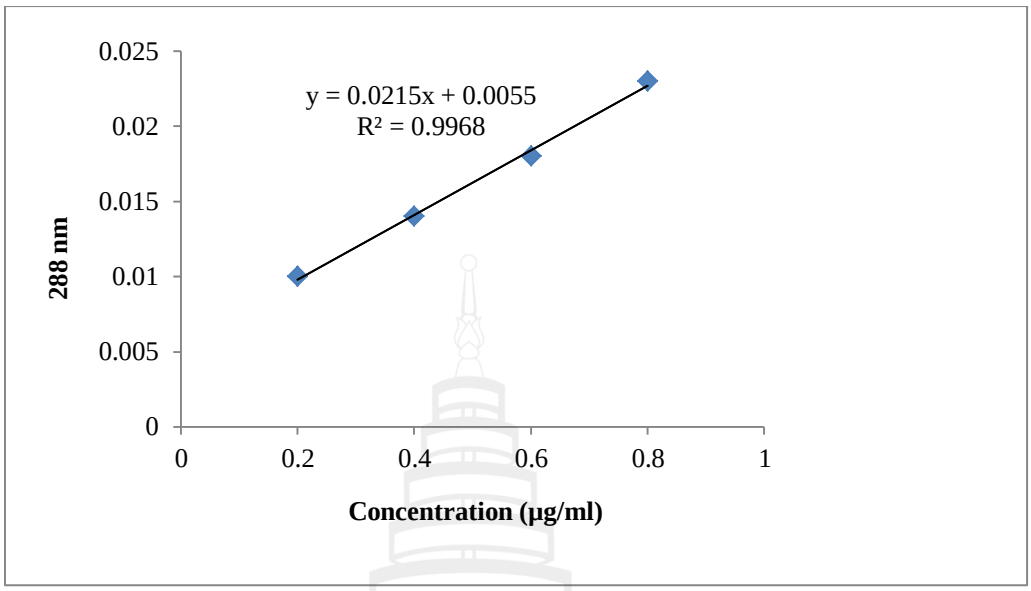

Figure 4.15 Calibration curve of alpha arbutin

4.3.2 Entrapment efficiency by indirect method

Total volume of washing solution was 15 ml. The absorbance was 0.008. The concentration of alpha arbutin in the waste water calculated from the calibration curve equation was 0.116 µg /ml. Then, the amount of alpha arbutin which was not able to be entrapped was 1.74 µg. Entrapment efficiency can be calculated with the following equation:

Entrapment efficiency (%) = 1.4 mg - 0.00174 mg/ 1.4 mg *100

1.4 mg is the total amount of alpha arbutin and

0.00174 mg is the unloaded amount of alpha arbutin

Entrapment efficiency (%) = 99.88

The entrapment efficiency can predict the capacity of amount of alpha arbutin as active agent in hydrogel nanoparticles. Besides, amount of alpha arbutin can be predicted if the ratio of hyaluronic acid to PEGDG is changed.

4.4 Swelling Studies

Swelling behavior (water uptake kinetic and equilibrium swelling) of the hydrogels was evaluated in distilled water by means of gravimetric measurements [21] using an analytical balance (Denver/TB214, USA). In particular, samples were immersed into distilled water (3 ml distilled water/ 4 mg of sample). The weight after swelling at 37 ˚C for 24 hours was 36 mg. The swelling ratio was calculated as follows:

Swelling ratio = 0.036 g -0.004 g /0.004 g = 8 g/g of hydrogel

0.036 g is the swollen sample weight

0.004g is the dried initial sample weight.

The HA- based hydrogel nanoparticles loaded alpha arbutin' swelling capacity was also analyzed. They showed swelling of 8 g/g of hydrogel. This study can indicate the successful to develop hydrogel nanoparticles as tropical delivery system. The swelling ratio provides a suitable system of hydrogels. Hydrogels in oil, w/o emulsion or o/w system will show slow, medium and fast alpha arbutin release, respectively because the more water in system, the more swelling ratio of hydrogels. In addition, route of penetration to deposit on the skin and how long the alpha arbutin release will be predicted because the less hydrogels' swelling, the smaller hydrogels' size. Moreover, this study will be useful to compare with other materials for delivery systems such as a study of cross-linked acrylic hydrogel for the controlled delivery of hydrophobic drugs in cancer therapy. The study showed that the release rate depended on the pH of the releasing medium (21.2 g/g of hydrogel) by polymerization of the acrylic acid with cross-linked polyethylene glycol diacrylate [21]. However, the swelling ratio depend on the purpose of study.

CHAPTER 5

CONCLUSION

Biocompatible and biodegradable HA-based hydrogel nanoparticles as novel drug delivery system were applied and developed to achieve localized alpha arbutin on skin. The inverse emulsion polymerization method was used to synthesize HA- based hydrogel nanoparticles and the loading alpha arbutin.

To characterize HA-hydrogel nanoparticles loading alpha arbutin then SEM, FT-IR, X-Ray diffraction and UV-Visible spectrophotometry were used. The size of hydrogel loading alpha arbutin was observed at dry state by using SEM and wetting state by zetasizer obtained ranged from 800-950 nm and 1496 nm, respectively. The FT-IR and X-ray diffraction analysis were used to approve composition of hydrogel nanoparticles. Due to remaining of HA in the hydrogel nanoparticles loading alpha arbutin, XRD peaks of HA and PEGDG were shifted. Those peaks indicated that the HA interacted with PEGDG to produce HA-base hydrogel nanoparticles. The alpha arbutin in the hydrogel was detected by using UV-Visible spectrophotometry after dispersing hydrogel nanoparticles loading alpha arbutin in distilled water for 24 hours. The result showed alpha arbutin was contained in the hydrogel nanoparticles.

The swelling study and the entrapment efficiency (%) of hydrogel nanoparticles loading alpha arbutin in distilled water were studied. The swelling behavior of hydrogel nanoparticles showed swelling of 8 g/g of hydrogel and the entrapment efficiency showed 99.88 %, respectively.

Overall, the results suggest that alpha arbutin HA-based hydrogel nanoparticles have potential as whitening agent and provide HA as NMF components on the skin. However, in this study the hydrogel nanoparticles have obtained a broad size distribution that may cause by the homogenization in the synthesis of hydrogel nanoparticles. The ultrasonication should be provided in homogenization for a narrow distribution size.

Moreover, the ratio of hyaluronic acid and PEGDG should be calculated properly to achieve crosslink polymerization.

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APPENDICES

APPENDIX A

CALIBRATION CURVE PREPARATION

Calibration curve of alpha arbutin preparation [19]

1. Lambda max of alpha arbutin

Before going to next step 0.1mg/ml of alpha arbutin solution was scanned wavelength between 250- 700 nm. by using UV-Vis spectroscopy to find out a lambda max.

2. Preparation of standard alpha- arbutin stock solution of 10000 µg/mL

Alpha- arbutin was weighed about 0.25 g into a 25 mL of volumetric flask, dissolve with distilled water, mixed and filled up to the volume with distilled water.

3. Preparation of standard solution for calibration curve

A series 200, 400, 600, 800 µl of mixed standard stock solution was pipette into 10 mL of volumetric flask and made up to the volume and mixed well. The concentrations of working standard solutions are 0.2, 0.4, 0.6 and 0.8µg/ml, respectively. Label as S_1, S_2, S_3 and S_4 were analyzed by UV-Vis spectroscopy.

APPENDIX B

RESEARCH PAPER

Preparation of hyaluronic acid- based hydrogel nanoparticles containing alpha arbutin

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Abstract

The hyaluronic acid (HA) and poly (ethylene glycol) diglycidyl ether (PEGDG) as biodegradable and biocompatible materials were used to produce hydrogel nanoparticles through chemical crosslinking. The hydrogel nanoparticles containing the alpha arbutin as whitening agent were characterized by mean of a scanning electron microscope (SEM) at dried state, an optical electron microscope image at wetting state. The particles size distribution was studied by using SEM and Zetasizer at dried state and wetting state, respectively. The components of hydrogel were confirmed by Fourier transform infrared spectroscopy (FT-IR) and X-ray diffractometry (XRD). The entrapment efficiency and swelling ratio were examined as well. The results showed that HA- based hydrogel nanoparticles loading alpha-arbutin obtained ranged from 800-950 nm at their dried states and above 1496 nm at wetting state. The peaks from Fourier transform infrared (FT-IR) at 1085 cm^{-1}, 1043 cm^{-1} can be attributed to stretching vibrations of C-O bond due to the chemically crosslinked hyaluronic acid and PEGDG as crosslinker. The confirmation by XRD was observed from the characteristic peaks of HA and hydrogel nanoparticles that both peaks are shown the same peak at nearby previous positions but the intensity were changed. Moreover, the broad peak changed to broader peak that because of shifting to lower intensity because of crosslinking polymerization. The Alpha arbutin in hydrogel nanoparticles was detected by using UV-Vis spectroscopy. HA- based

hydrogel nanoparticles containing alpha arbutin were dispersing in distilled water for 24 hours showed swelling of 8 g/g of nanogel and the entrapment efficiency was almost completely (99.88 %), respectively.

Keywords HA-based hydrogel nanoparticles/Crosslinking polymerization/ Alpha arbutin

INTRODUCTION

Due to arbutin is one of effective whitening agents that highly hydrophilic and hydroscopic substance but the formidable barrier property of stratum corneum and high hydrophilicity of arbutin make it difficult to permeate through the skin and reach to its site of action (i.e. melanocytes)[1]. In addition, arbutin is a thermodegradable substance [2]. Thus, there were several studies that provided for solutions.

Hydrogels are hydrophilic network polymers which are glassy in the dehydrated state and swollen in the presence of water to form an elastic gel. Although hydrogels are of either natural or synthetic origin, they are the covalently crosslinked synthetic hydrogels that have been gaining increasing popularity in various biomedical applications [3]. Previous study demonstrated that the hyaluronic acid-based hydrogel nanoparticles were synthesized via a crosslinking reaction between hyaluronic acid (HA) and polyethylene glycol [4]. These components were biodegradable and biocompatible with the skin. The study showed that the hyaluronic acid- based hydrogel nanoparticles could penetrate into shallow region (horny layer) of the skin. The study is beneficial to a tropical delivery system due to penetration of the particles are on the surface of the skin. Thus, the aim of this study is to prepare hyaluronic acid- based hydrogel nanoparticles containing alpha-arbutin and to study the physicochemical properties of the obtained arbutin nanoparticles including swelling studies and entrapment efficiency.

MATERIALS

Hyaluronic acid (HA) was obtained from M.C.Biotec.Inc, China, Poly (ethylene glycol) diglycidyl ether (PEGDG) was obtained from Aldrich, Dodecane, NaOH, Acetone, Citric acid were also obtained from Aldrich. Polyethylene glycol 30,

Arlacel P135, was obtained from Croda International. Alpha arbutin was purchased from Thornhill advances research Inc.

METHODS

Hyaluronic acid –based Hydrogel nanoparticles containing alpha arbutin

Nanoparticles loading alpha arbutin were prepared by the inverse emulsion polymerization method [4]. Dissolving hyaluronic acid (0.34 g), PEGDG (0.79 g) and alpha arbutin (1.4 mg) in 0.1 N sodium hydroxide aqueous solution (11.25 ml) for aqueous phase. Arlacel P 135 (1.13g) as solubilizer dissolved in dodecane (27.16 ml) as oil phase. Then, the aqueous solution was added slowly to oil phase. The emulsion was homogenized at 6000 rpm for 13 minutes by homogenizer. After that the emulsion was transferred into a round bottom flask. Heating the w/o inverse emulsion at 60 ◦C and maintaining the temperature while stirring for 1 hour. Then, the emulsion was left at room temperature, and adding acetic acid (0.065 ml) to neutralize the aqueous solution. For a post-crosslinking reaction was left at room temperature for 2 days. After synthesis, the emulsion was transferred slowly to acetone and hydrogels were collected and dried in hot air oven at 90 ^{o}C for 24 hours. The polymerization of the hyaluronic acid with cross-linked PEGDG was characterized by SEM, optical microscope, zetasizer, Fourier transforms infrared (FT-IR) spectroscopy and XRD.

Swelling studies

Swelling behavior (water uptake kinetic and equilibrium swelling) of the hydrogels was evaluated in distilled water by means of gravimetric measurements using an analytical balance (Denver/TB214, USA). In particular, samples were immersed into distilled water (3 ml distilled water/ 4 mg of sample) and weight after was kept at 37 ^{o}C for 24 hours. The swelling ratio was calculated as follows:

$$\text{Swelling ratio} = Ws_{(g)}\text{-}Wd_{(g)}/Wd_{(g)}*100$$

Ws is the swollen sample weight

Wd is the dried initial sample weight.

Entrapment efficiency by indirect method [5]

Total volume of washing solution was kept and detected at 288 nm by UV-Vis spectroscopy. Entrapment efficiency can be calculated with the following equation:

$$\text{Entrapment efficiency (\%)} = W_A\text{-}W_a/W_A*100$$

W_A is the total amount of alpha arbutin and

W_a is the amount of remaining alpha arbutin

RESULTS

Characterization of hydrogel nanoparticles loaded alpha arbutin

The purpose of this study is to develop novel hydrogel nanoparticles- loaded alpha arbutin powder as whitening agent. It was successfully by preparing the inverse emulsion polymerization method. In this study, we chose HA and PEG as components of hydrogel nanoparticles. Both are biocompatible and highly water soluble, and HA is a natural polysaccharide and which take up tremendous amount of water. Therefore, we expect that the combination of the two chemical compounds might produce suitable hydrogel nanoparticles as delivery carriers. Moreover, HA is a one of the NMF (natural moisturizing factor) that it might help boost up moisturizer on the skin during the releasing alpha arbutin. The results show that, the crosslinking reaction between two biopolymers is completely to be a shelter for alpha arbutin as purpose. The crosslinking polymerization provided white pieces with naked eyes and small sphere particles by SEM (x1000). Furthermore hydrogels appeared clear and small particles under optical microscope after dispersing in distilled water for 24 hours. The hydrogel particles'size was studied by SEM at dried state and zetasizer at wetting state the result showed that most of nanoparticles obtained ranged from 800-950 nm and above 1496 nm, respectively.

The hyaluronic acid which composed of hydrogel was confirmed by mean of FT-IR. The CH stretching vibration spectrum of hyaluronic acid and PEGDG shifted to 2851 and 2924 nm^{-1}, respectively. The spectrum of C=N bonding at 2308 cm^{-1} is able to confirm hyaluronic acid component. The peak at 1085 cm^{-1}, 1043 cm^{-1} can be attributed to stretching vibrations of C-O stretching due to crosslinking polymerization at ether

group of PEGDG and carboxyl group of HA. The FT-IR spectrum confirmed that the hydrogel composed of PEGDG and hyaluronic acid.

The confirmation by XRD was observed from the characteristic peaks of HA and hydrogel nanoparticles that both peaks are shown the same peak at nearby previous positions but the intensity were changed. Moreover, the broad peak changed to broader peak that because of shifting to lower intensity because of crosslinking polymerization.

Entrapment efficiency

The amount of alpha arbutin which was not able to be entrapped was 0.00174 mg. The entrapment efficiency of the cross-linked polymers was 99.88%.

Swelling studies

The swelling of the polymers was studied with gravimetric analysis. The hydrogel nanoparticles loaded alpha arbutin showed swelling of 8 g/g of nanogel

DISCUSSION

Arbutin, white crystalline powder, is one of effective whitening agents that highly hydrophilic and hydroscopic substance but the formidable barrier property of stratum corneum and high hydrophilicity of arbutin make it difficult to permeate through the skin and reach to its site of action (i.e. melanocytes)[1]. HA-based hydrogel nanoparticle-mediated drug delivery system has proved excellent solution to these problems [3].

Biocompatible and biodegradable HA-based hydrogel nanoparticles as novel drug delivery system were applied and developed to achieve localized alpha arbutin on skin. The inverse emulsion polymerization method was used to synthesize HA-based hydrogel nanoparticles and the loading alpha arbutin.

To characterize HA-hydrogel nanoparticles loading alpha arbutin then SEM, Zetasizer, FT-IR, X-Ray diffraction and UV-Visible spectrophotometry were used. The size of hydrogel loading alpha arbutin obtained ranged from 800-950 nm and above 1496 nm at dried state and wetting state, respectively. The FT-IR and X-ray diffraction analysis were used to approve composition of hydrogel nanoparticles. Due to remaining of HA in the hydrogel nanoparticles loading alpha arbutin, XRD peaks

of HA and PEGDG were shifted. Those peaks indicated that the HA interacted with PEGDG to produce HA-base hydrogel nanoparticles.

The swelling study and the entrapment efficiency (%) of hydrogel nanoparticles loading alpha arbutin in distilled water were studied. The swelling behavior of hydrogel nanoparticles showed swelling of 8 g/g of hydrogel and the entrapment efficiency showed 99.88%, respectively. This study can indicate the successful to develop hydrogel nanoparticles as tropical delivery system. The swelling ratio provides a suitable system of hydrogels. Hydrogels in oil, w/o emulsion or o/w system will show slow, medium and fast alpha arbutin release, respectively because the more water in system, the more swelling ratio of hydrogels. In addition, route of penetration to deposit on the skin and how long the alpha arbutin release will be predicted because the less hydrogels' swelling, the smaller hydrogels' size. The entrapment efficiency can predict the capacity of amount of alpha arbutin as active agent in hydrogel nanoparticles.

CONCLUSIONS

Overall, the results suggest that alpha arbutin HA-based hydrogel nanoparticles have potential as whitening agent and provide HA as NMF components on the skin. However, in this study the nanoparticles obtained a broad size distribution that might cause the homogenization in the synthesis of hydrogel nanoparticles should been remained speed and time or the sonication should be used to reduce smaller and narrow size.

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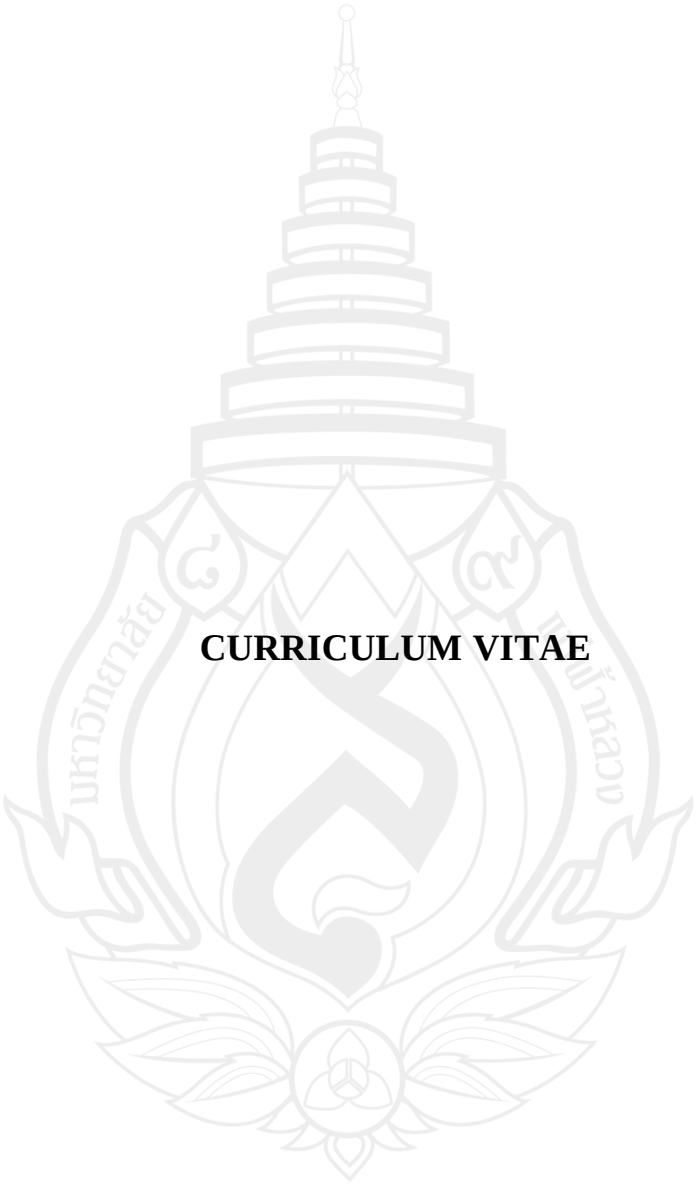

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