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[54] **CROSS-LINKED GELS OF HYALURONIC ACID AND PRODUCTS CONTAINING SUCH GELS**

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[51] **Int. Cl.**[4] **C08F 8/00**

[52] **U.S. Cl.** **524/29;** 536/4.1; 524/27; 514/781

[58] **Field of Search** 524/27, 29; 536/4.1

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,357,784	12/1967	Kasper	8/129
4,141,973	2/1979	Balazs	536/55.1
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4,487,865	12/1984	Balazs et al.	524/29
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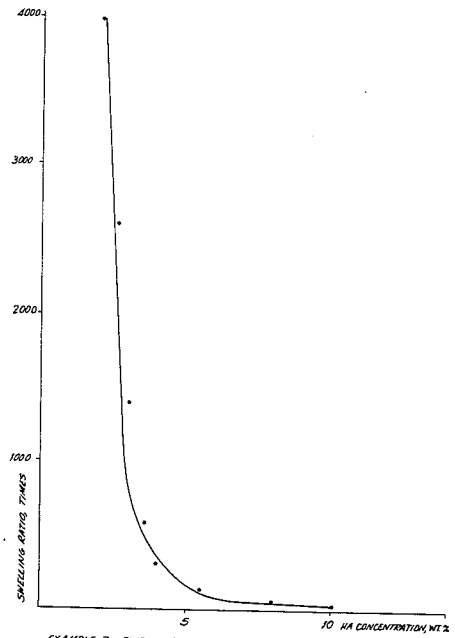
[57] **ABSTRACT**

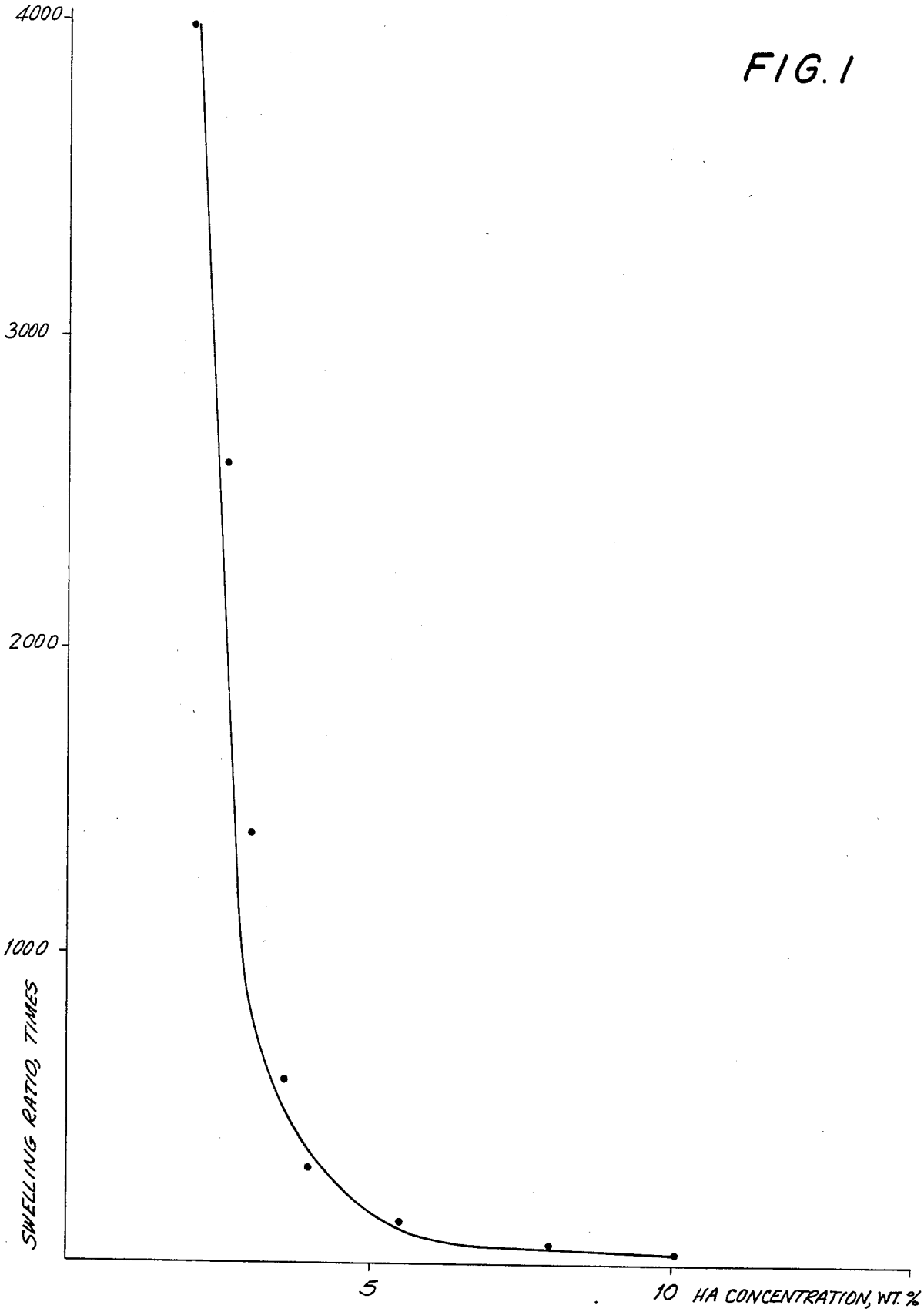
Disclosed are cross-linked gels of hyaluronic acid, alone or mixed with other hydrophilic polymers and containing various substances or covalently bonded low molecular weight substances and processes for preparing them. These products are useful in numerous applications including cosmetic formulations and as drug delivery systems.

28 Claims, 2 Drawing Figures



EXAMPLE 3. SWELLING RATIO OF CROSS-LINKED HA GELS AT DIFFERENT CONCENTRATIONS OF HA IN STARTING SOLUTIONS (HA/DVS WEIGHT RATIO ABOUT 1:1, ROOM TEMPERATURE, 1 HOUR).

FIG. 1



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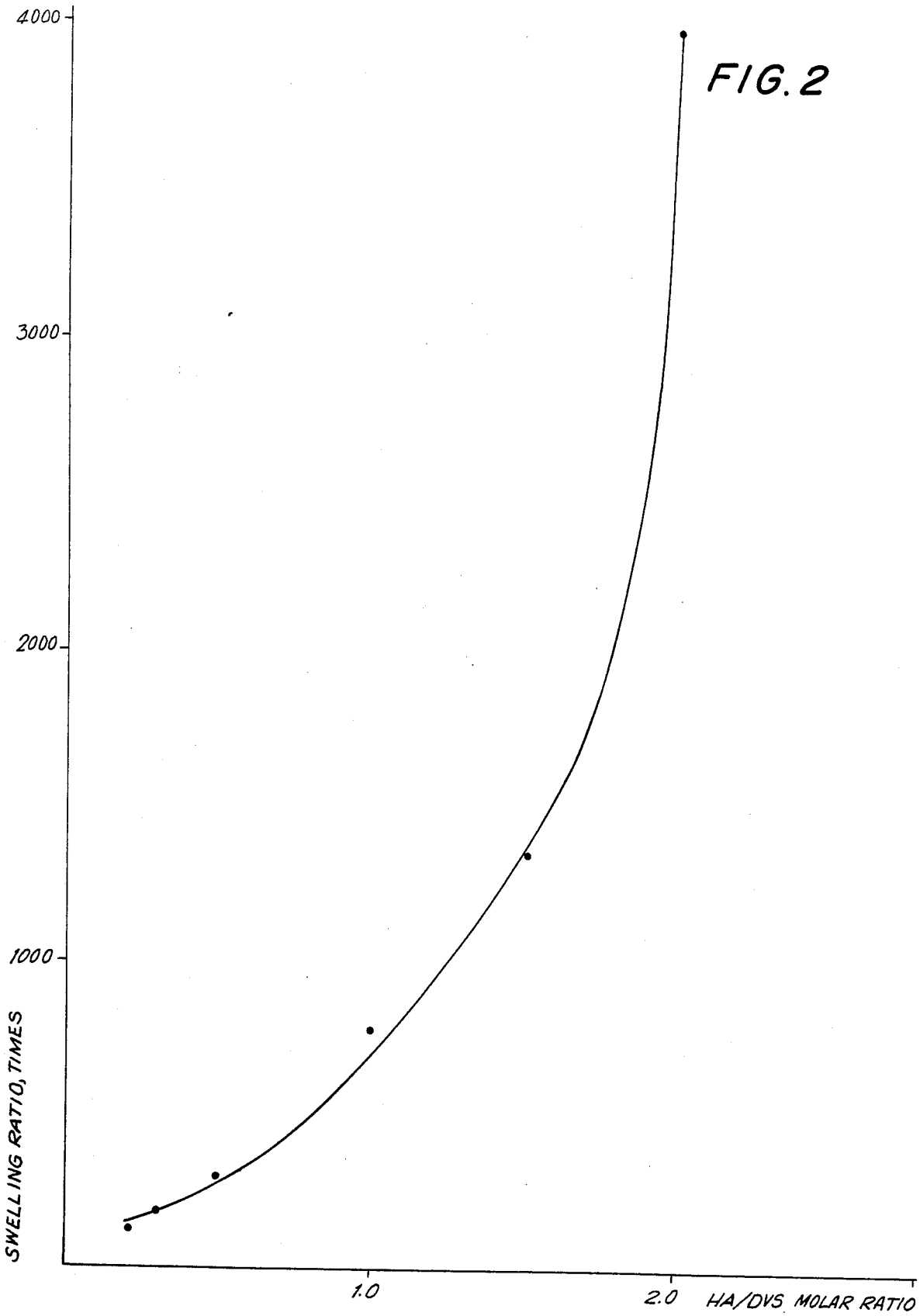

FIG. 2

EXAMPLE 4. SWELLING RATIO OF CROSS-LINKED HA GELS AT DIFFERENT HA/DVS MOLAR RATIOS (HA CONCENTRATION IN STARTING

CROSS-LINKED GELS OF HYALURONIC ACID AND PRODUCTS CONTAINING SUCH GELS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to gels and mixed gels of hyaluronic acid (HA), formulations containing them and methods for preparing them.

2. The Prior Art

Hyaluronic acid is a well known, naturally occurring polysaccharide containing alternating N-acetyl-D-glucosamine and D-glucuronic acid monosaccharide units linked with $\beta 1 \rightarrow 4$ bonds and the disaccharide units linked with $\beta 1 \rightarrow 3$ glycoside bonds. Hyaluronic acid usually occurs as the sodium salt. The molecular weight of HA is generally within the range of 50,000 up to 8×10^6 and even higher.

The prior art describes the cross-linking of HA with the use of 1,2,3,4-diepoxybutane in alkaline medium at 50° C. (T. C. Laurent, K. Hellsing, and B. Gelotte, Acta Chem. Scand. 18 [1984], No 1, 274–5). The product obtained by that method is a gel which substantially swells in water.

It is also known that divinyl sulfone (DVS) is used for cross-linking polysaccharides, especially cellulose (U.S. Pat. No. 3,357,784).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graphical representation of the experimental data set forth in Example 3 below; and

FIG. 2 is a graphical representation of the experimental data set forth in Example 4 below.

SUMMARY OF THE INVENTION

In one aspect thereof, the present invention provides highly swollen gels of cross-linked hyaluronic acid.

In another aspect, the invention provides mixed cross-linked gels of hyaluronic acid and other hydrophillic polymers.

In yet another aspect, the invention provides cross-linked gels of hyaluronic acid and other polymers filled with various substances.

In still another aspect, the invention provides cross-linked gels of hyaluronic acid containing low molecular weight substances covalently attached to the macromolecules.

In still yet another aspect, the invention provides various formulations containing cross-linked hyaluronic acid gels.

Finally, the invention provides the methods of preparing the products of the invention.

The present invention is based on the observation that divinyl sulfone (DVS) reacts readily with HA in aqueous alkaline solutions at room temperature, i.e., about 20° C., thereby providing cross-linked HA gels. As used herein, the term HA means hyaluronic acid and its salts such as the sodium, potassium, magnesium, calcium, etc. salts. These gels swell in water and water containing media. The swelling ratio depends upon the degree of cross-linking of the gel. We have found that the degree of cross-linking can be controlled by changing several factors including the molecular weight of the HA, its concentration in the reaction mixture, the alkali concentration and the polymer/DVS ratio. The reaction is very fast and in most cases a strong gel can be obtained in several minutes. The swelling ratio of these gels can be from 20 up to 8000, and more, depending upon the reaction parameters.

It has also been found that the swelling ratio of cross-linked HA gels is substantially greater than the swelling ratio of cross-linked gels of other polysaccharides obtained under the same reaction conditions. This can probably be explained by the unique nature of HA (as compared to other polysaccharides) and its water solutions. We have found that in water, a large molecule of HA forms a very flexible, long random coil which takes up an extremely large volume in the solution. For example, the specific volume of a hydrated HA molecule in a physiological salt solution is about $2\text{–}6 \times 10^3$ ml/g. That means that in a quite low concentration water solution of HA, a steric exclusion phenomenon occurs which will substantially affect not only the physico-chemical properties of the solution, but the reaction of the HA with low molecular weight substances as well. In other words, the nature of the HA solutions affects the degree of cross-linking and the behavior of the cross-linked gel, in a manner quite unlike anything that occurs with other polysaccharides.

We have also found that this unique property of HA to give highly swollen cross-linked gels can be used to effect modification of the properties of cross-linked gels made of mixtures of HA with other hydrophillic polymers. These polymers include other polysaccharides, synthetic and natural, such as hydroxyethyl cellulose, carboxymethyl cellulose, xanthan gum, chondroitin sulfate, heparin, proteins of various types, such as collagen, elastin, albumin, a globulin, etc., sulfated proteins such as keratin sulfate and sulfated aminoglycosaminoglycans, synthetic water-soluble polymers, such as polyvinyl alcohol and its co-polymers, co-polymers of poly-(hydroxethyl)methacrylate and the like. In other words, any polymer soluble in water or water alkaline solutions and containing groups capable of reacting with DVS, namely, hydroxyl, amino or sulfyhydryl groups, can be used to obtain highly swollen cross-linked mixed gels of HA.

We have further found that useful products can easily be obtained by carrying out the cross-linking reaction of HA in the presence of low-molecular weight substances containing reactive groups of the mentioned types.

Another type of material according to the present invention is a cross-linked hydrophillic gel filled with various water insoluble substances including hydrocarbons, such as petrolatum; an oil or fat such as beeswax, conconut oil or lanolin, pigments, such as kaolin, ferric oxide; insoluble dyes, polymers, such as polyethylene, polyetrafluro ethylene, etc. In this type of product fine particles of a filler are immobilized in a gel network or in what we call a "polymer cage". This latter product can be very useful for several purposes which will be discussed in more detail below.

DESCRIPTION OF THE PREFERRED EMBODINEMT

The processes by which the hereinabove described products are obtained will now be discussed in detail.

In order to obtain a cross-linked HA gel, a sample of sodium hyaluronate or hyaluronic acid from any source is dissolved in dilute alkaline solution. The molecular weight of HA can be from 50,000 up to 8×10^6 and even higher. The molecular weight affects the reation—the higher the molecular weight the greater the possibility to obtain a cross-linked gel.

The alkali concentration in the reaction mixture can be from 0.005M to 0.5M and higher. The lower limit is dictated by the necessity to have the pH of the medium not lower than 9 and the upper limit by the hydrolysis of HA in an alkaline solution. Usually, a decrease in alkali concentration results in gels with a greater swelling ratio, probably because a small amount of DVS takes part in the cross-linking reaction.

The concentration of HA in the starting solution can vary from 1% by weight up to 8% by weight and higher. When the concentration is below the lower limit, a cross-linked gel cannot be obtained even at a low HA/DVS ratio. When the concentration is too high, the solution becomes so viscous that it is difficult to handle it. The HA concentration substantially affects the swelling behavior of the gels (FIG. 1). It was found that the shape of the curve for the swelling ratio—the HA concentration dependence is essentially the same for various HA/DVS ratios but the lower this ratio (i.e., more DVS in the mixture), the less the swelling ratio of the cross-linked gel for the same concentration of HA in the starting mixture.

We have found that HA/DVS in the reaction mixture is another parameter which can be conveniently used to control the swelling ratio of the cross-linked HA gel. An increase in the ratio results in highly swollen soft gels (the swelling ratio is about 4000 and higher) whereas hard and less swollen gels are obtained when this ratio is decreased. In general, the HA/DVS weight ratio can be from 15:1 to 1:5 and lower.

The cross-linking reaction is usually carried out at room temperature, i.e., about 20° C., but it can be performed at a lower or higher temperature, if desired. However, it should be kept in mind that HA can degrade relatively rapidly in alkaline solutions at elevated temperatures and, if such degradation occurs, the decrease in MW can affect the properties of the obtained gels.

The cross-linking reaction is relatively fast and strong gels are formed usually in several minutes when the HA concentration is high enough and the HA/DVS ratio is low. But even at low HA concentration in the reaction mixture, the gel formation starts usually 5–10 minutes after addition of DVS. We have found that in most cases one hour is enough for completion of the cross-linking reaction.

Another method of controlling the swelling ratio of cross-linked HA gels involves adding neutral salt to the reaction mixture. We have found that the swelling ratio of the gels obtained in the presence of water soluble neutral salts, such as the chlorides, sulfates, phosphates and acetates of alkali metals, decreases with the increase of salt concentration. A salt can be used in concentration up to 20 wt. % and higher, depending upon the nature of the salt and its effect on the solubility of HA in the reaction mixture.

To obtained cross-linked gels of other hydrophillic polymers the same reaction conditions as for HA can be used. The swelling ratio of these gels can be conveniently controlled by incorporating HA into the gel structure. When the mixed gels are obtained, the composition of the polymer mixture can vary over a broad range depending on the swelling ratio of the cross-linked gel desired. The preferred content of HA in the mixture is from 5 to 95 wt. %.

Cross-linked gels of HA or other polymers or mixed cross-linked gels filled with inert substances are obtained by incorporating these substances into the reaction mixture before the addition of DVS. These inert substances are, preferably, water-insoluble liquids or solid substances. Examples of such substances are petrolatum and kaolin. To obtain a filled cross-linked gel, a chosen substance (based on a consideration of the desired properties of the gel) is emulsified or dispersed in an alkaline solution of HA or other polymer or mixture of HA with other polymer or polymers and DVS is added to the mixture. The amount of DVS and the other parameters of the reaction are selected depending upon the desired properties of the gel. The relative amount of filler in the gel can vary over a broad range and is from 1 to 95 wt. % calculated on the total amount of polymers and filler, preferably from 5 to 90 wt. %.

Cross-linked gels containing low molecular weight substances such as drugs, dyes and others covalently attached to the macromolecular network are obtained, preferably by incorporating the named substances into an HA or HA and other polymers solution before the addition of DVS. An example of such a substance is carminic acid, an FDA approved substance for use in food and drug preparations.

It is probably the presence of a glucosidic moiety of the carminic molecule which takes part in the cross-linking reaction with DVS. It should be understood that a great number of substances can be used to obtain a modified cross-linked gel of this type. The only essential feature of these substances is that they contain chemical groups with active hydrogen atoms reactive to DVS. The amount of such low molecular weight substances which can be used in the reaction depends upon the desired level of that substance in the gel. This amount can be in the range of from 1 to 99 wt. % as calculated on polymer content in the gel, preferably, 5 to 90 wt. %.

The cross-linked HA and mixed gels obtained according to the present invention can be used for many purposes. We have found that these highly swollen gels are very useful in cosmetic formulations and can be considered as water-retaining and water-delivering ingredients in these formulations.

As HA is known to be a biologically tolerable polymer in the sense that it does not cause any immune or other kind of response when introduced into a human body, the cross-linked HA gels can be used for various medical applications. The cross-linked gels modified with other polymers or low molecular weight substances can be used as drug delivery devices. For example, we have found that heparin introduced in a cross-linked HA gel retains its antithrombogenic activity.

We have also found that cross-linked gels of HA can slow down the release of a low molecular weight substance dispersed therein but not covalently attached to the gel macromolecular matrix.

The domain of the cross-linked hyaluronic acid (alone or co-polymerized with other polyanionic or neutral polymers) forms a molecular cage. In this cage, hydrophilic or hydrophobic molecules of various pharmacological or biological activity can be dispersed. Thus, the cage constitutes a depot for these substances of various molecular size. The substances contained in the domain of the molecular cage will be delivered into the environment by diffusion. The delivery process is controlled by such factors as the exclusion volume effect and the pore size of the molecular cage and by the molecular interaction between the polymeric network and the substance contained therein. Thus, the molecular cage forms a depot for the controlled delivery of drugs or other substances to the skin or other tissues.

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