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(54) Title: METHOD OF USE OF CARBOXYLATED POLYSACCHARIDES TOPICALLY ON THE EYEBALL

(57) Abstract: The use of chemically modified dicarboxy polysaccharides for the topical treatment of the eyeball are described. The modified polysaccharides provide a carrier in solutions for the treatment of the eyeball to obtain a timed release.

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METHOD OF USE OF CARBOXYLATED POLYSACCHARIDES TOPICALLY ON THE EYEBALL

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional application Serial No. 60/569,723, filed May 10, 2004.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not Applicable

STATEMENT REGARDING GOVERNMENT RIGHTS

[0003] Not Applicable

BACKGROUND OF THE INVENTION

(1) Field of the Invention

[0004] The present invention relates to the use of groups chemically modified polysaccharides which have carboxylic acid on the eyeball. The composition provides a timed release of the medicament on the outside of the eyeball.

(2) Description of the Related Art

[0005] The prior art describes various compositions derived from natural carbohydrates for use as carriers and processes for the preparation of modified carbohydrates, particularly cellulose. Natural polymers and gums have been used in pharmaceutical formulations of sustained-release carriers, and modified celluloses, carboxy methylcellulose (CMC) and modified methyl cellulose (MMC) are found in a large number of

formulations as viscosity enhancers. Because of their wide acceptance of these modified natural polymers, pharmaceutical companies are interested in the use of modified natural polymers for their drug delivery systems. Natural polymers with gelling properties that have been successfully used in topical formulations include gellan gum and carrageenans. Topical formulations with gelling properties afford increased ocular bioavailability of certain drugs.

[0006] The literature and patents show that much of the focus is centered around natural polymers. GELRITE, a registered trademark of Monsanto, is used by Merck (Radway, New Jersey) in a preparation of timolol, TIMOPTIC XE. This is the only known *in situ* gelling drug delivery system currently on the market. It is a low-acetyl gellan gum which would have a structure similar to Figure 3 and can ionically crosslink in the presence of a divalent cation such as calcium. Rozier et al (International Journal of Pharmaceutics 57:163-168 (1989) has shown that in *in vivo* testing GELRITE behaved similar to HEC (hydroxyethycellulose), a known viscosity enhancer. It significantly reduced intraocular pressure over the HEC and this was determined to be caused by an increased residence time at the surface of the eye.

[0007] Another natural gel-forming polysaccharide is alginate. Cohen et al (Opthalmic Delivery System. US, Teva Pharmaceutical Industries (1998)) describe an alginate system that gels in the presence of calcium ions in the eye. Alginate is a mixture of guluronic and mannuronic acids. They suggest using a mixture with the guluronic acid concentrations higher than 65% to form a suitable gel. When testing

pilocarpine, a common glaucoma treatment, the alginate formulated system demonstrated a correlation between the gelation capability of the alginate formulation and the speed at which it occurs and the sustained release properties. It was also claimed that there was excellent ocular tolerance in the test rabbits; even though redness of the conjunctivae was reported for 1-2 hours after instillation of the drops.

[0008] A final natural polysaccharide that can form gels *in situ* is pectin. The pectin was isolated from Aloe Vera, which contains a higher galacturonic acid ration will form a gel when subjected to mono- or divalent ions at a low pectin of concentration of 0.25% w/v. It will also form a gel in the presence of small organic compounds, proteins, nucleic acid, and live cells.

[0009] GELFOAM, a structured matrix of gelatin, has been studied for the release of pilocarpine. The matrix is a structured water-insoluble sponge prepared from purified pork skin gelatin that will biodegrade. Because this simple matrix released most of the drug within 15 minutes, retardants had to be added. This matrix embedded in cetyl ester wax demonstrated zero-order release kinetics while the matrix impregnated with polyethylene glycol 400 monostearate exhibited close to first-order kinetics. The results show that gelatin itself does not provide for good sustained release. The following table summarizes work in ocular drug delivery systems.

Table 3. Ocular drug delivery systems

Matrix Material	Method of Action	Author
Natural Polymers		
Alginate	Ionic concentration	Cohen (Cohen 1998)
Gellan Gum	Ionic concentration	Rozier (Rozier, Mazuel et al. (1989)
Pectin	Ionic concentration	NI (Ni and Yates (2002)
Gelatin	Not *in situ* gelation	Nadkarni (Hadkarni and Yalkowsky (1993)
Cyclodextrins	Not *in situ* gelation	
Synthetic Polymers		
Poloxamer	Temperature change	Lin (Lin and Sung 2003)
Pluronic	Temperature change	Lin (Lin and Sung 2003)
Carbopol	pH change	Lin (Lin and Sung 2003)
Cellulose acetophthalate	pH change	Gurney (Gurney 1986)

[0010] Many synthetic polymers have been tested for sustained release in the eye. While they have the advantage of being engineered to specific applications, their breakdown products are not always known, which can lead to extended FDA testing. The prior art has described a formulation approach of combining Carbopol and Pluronic. Carbopol is a high molecular weight carboxy vinyl polymer and Pluronic is a class of block copolymers containing polyoxyethylene and polyoxypropylene. This formulation claims to be free-flowing at non-physiological conditions (pH 4.0 and 25°C) , but forming a gel at physiological conditions (pH 7.4 and 37°C). A disadvantage to this system is the high amount of Pluronic (14%) required for optimal gel formation. Again there are many disadvantages of synthetic polymers including high

polymer concentration, irritancy and potentially harmful breakdown products.

OBJECTS

[0011] It is an object of the present invention to provide modified polysaccharides for topical use on the eyeball. This and other objects will become increasingly apparent by reference to the following description.

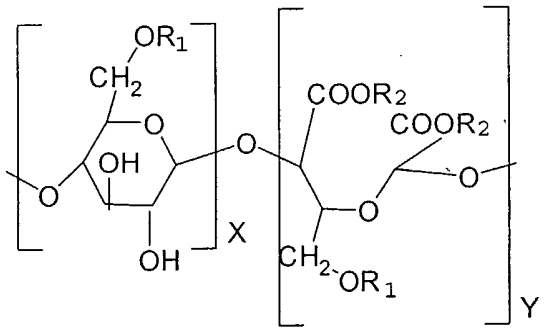
SUMMARY OF THE INVENTION

[0012] The present invention relates to a method for providing a topical timed release of a medicament for the eyeball of an animal including humans or other mammals in need thereof, which comprises: providing a composition which comprises: a medicament; and a chemically modified polysaccharide (CMP) comprising linked saccharide rings with ring opened saccharide units at C_2 and C_3 bond and containing carboxylic acid moieties or water dispersible salt as a random copolymer wherein the CMP is water dispersible to form a clear solution as a time release adjuvant for the medicament; and topically providing the medicament on the eyeball to provide the timed release.

[0013] The present invention particularly relates to a pharmaceutical composition for topical treatment of the eyeball which comprises: a medicament for the eyeball; and a chemically modified polysaccharide (CMP) comprising linked saccharide rings with ring opened saccharide units at C_2 and C_3 bond and containing carboxylic acid moieties or water dispersible salt as a random copolymer wherein the CMP is water

dispersible to form a clear solution as a time release adjuvant for the medicament onto the eyeball.

[0014] Preferably in the CMP all or portions of C_2 and C_3 is a hydroxyl group other than at units which have carboxylic acid group. Most preferably, in the CMP both C_2 and C_3 are carboxylic acid groups. Preferably the CMP is a copolymer of linked units of the formula:



wherein ring opened units in the copolymer are between 10 to 90 mole percent, wherein R_1 is H or COOR where R is alkyl or aryl, and wherein R_2 is H, alkyl or an aryl group containing 1 to 12 carbon atoms. Preferably the animal is a mammal. Preferably the mammal is human.

[0015] A pharmaceutical composition for topical treatment of the eyeball which comprises:

(a) a medicament for the eyeball; and

(b) a chemically modified polysaccharide (CMP) comprising linked saccharide rings with ring opened saccharide units at C_2 and C_3 bond and containing carboxylic acid moieties or water dispersible salt as a random copolymer wherein the CMP is water dispersible to form a clear solution as a time release adjuvant for the medicament onto the eyeball.

BRIEF DESCRIPTION OF DRAWINGS

[0016] Figure 1 shows the structures of starch and cellulose.

[0017] Figure 2 shows a reaction scheme oxidation of starch.

[0018] Figure 3 shows a copolymer starch structure.

[0019] Figure 4 shows 3D structure of 50% dicarboxy starch.

[0020] Figure 5 shows possible structures of periodate-oxidized starch. 1) free aldehyde 2) hydrated aldehyde 3) hemialdol 4) hemiacetal;

[0021] Figure 6 shows a FTIR Comparison of oxidation methods.

[0022] Figure 7 shows a FTIR of a possible oxidized hemialdol structure.

[0023] Figure 8 is a graph showing carboxyl content versus periodate ratio.

[0024] Figure 9 is a graph showing periodate oxidation of cellulose kinetics.

[0025] Figure 10 is a graph showing periodate oxidation of starch kinetics.

[0026] Figure 11 is a graph showing a comparison of actual and theoretical kinetic data.

[0027] Figure 12 is a graph showing the relationship between the periodate ratio and the yield.

[0028] Figure 13 is a graph showing the relationship between the periodate ratio and the final material acid content.

[0029] Figure 14 is a graph showing the relationship between the periodate ratio and the dispersibility of the material.

[0030] Figure 15 is a graph showing optimization of the periodate concentration.

[0031] Figures 16A to 16E show ESEM scanning electron microscope images of starch (Figures 16A to 16C), air dried dicarboxy starch (Figure 16D) and hydrated dicarboxy starch (Figure 16E).

[0032] Figure 17 shows large-scale reaction scheme.

[0033] Figure 18 shows structure of ofloxacin.

[0034] Figure 19 shows UV-VIS spectrum of ofloxacin.

[0035] Figure 20 shows calibration curve for ofloxacin.

[0036] Figure 21 shows comparison of dicarboxy starch to GELRITE.

[0037] Figure 22 shows drug release of various materials.

[0038] Figure 23 shows release profiles using USP dissolution system.

[0039] Figure 24 shows release with varying drug concentration.

[0040] Figure 25 shows square root time dependence of the ofloxacin.

[0041] Figure 26 shows calculation of diffusion coefficient.

[0042] Figure 27 shows stir plate dissolution system.

[0043] Figure 28 shows membrane support (above) and dissolution bath for drug release studies.

DESCRIPTION OF PREFERRED EMBODIMENTS

[0044] The present invention provides a flexible, water dispersible, biocompatible material for the topical treatment of the eyeball. It was determined that engineering carboxy groups onto a natural polysaccharide backbone was used to provide a water dispersible biocompatible material. Starch and cellulose were chosen as the polysaccharide backbone because of their abundance and their current acceptance in other pharmaceutical applications. The engineering of the carboxy starch with rigid sugar units and flexible chain segments carrying carboxy groups and being water soluble or dispersible is novel. Application of these oxidized polysaccharides to drug delivery systems for the eye is also novel. Different methods of oxidation are known, including the use of sodium periodate, hypochlorite, or ozone. All of these methods can be used separately or combined until the desired material properties are achieved. Oxidation by sodium periodate was preferred research because it has the best method to control the position and extent of oxidation.

[0045] Cellulose and starch both consist of repeating glucose units with only the glycosidic bond differing as seen in Figure 1. The oxidation methods could be applied to either structure, although there are differences in the kinetics because of the structure of the materials. Starch is composed of amylose that forms a helical structure. When the material is hydrated, the helices open and water can penetrate the material easily. Cellulose, on the other hand, forms a tight crystalline structure that is not as easily hydrated.

[0046] In the periodate method, the starch/cellulose ring

is opened between the C-2 and C-3 using $NaIO_4$ in the first step (Floor, M., L.P.M. Hofsteede, W.P.T. Groenland, L.A. Th.Verhaar, A.P.G. Kieboom, H. van Bekkum: Preparation and calcium complexation of oxidized polysaccharides. II: Hydrogen peroxide as co-reactant in the chlorite oxidation of dialdehyde glucans. Recl. Trav. Chim. Pays-Bas. (1989), 108, 384-392) which forms an aldehyde structure. Secondly, the dialdehyde is oxidized using any oxidizing agent (i.e. NaOCl) and carboxyl groups will be formed at the C-2 and C-3 (see Figure 2.

[0047] In this method, by controlling the amount of ring opening, the total amount of carboxylation can be controlled. Floor (Floor, M., L.P.M. Hofsteede, W.P.T., Groenland, L.A. Th.Verhaar, A.P.G. Kieboom, H. van Bekkum: Preparation and calcium complexation of oxidized polysaccharides. II: Hydrogen peroxide as co-reactant in the chlorite oxidation of dialdehyde glucans. Recl. Trav. Chim. Pays-Bas. (1989), 108, 384-392) described a process where the second step oxidation uses hydrogen peroxide as an inexpensive HOCl scavenger that will reduce the HOCl. The reaction is as follows:

$$R\text{-}CHO + ClO^{-2} \rightarrow R\text{-}COO^{-} + HOCl$$

$$HOCL + H_2O_2 \rightarrow HCl + H_2O + O_2$$

[0048] This was an important improvement over previous methods which used ClO^{-2} as a scavenger. Besides being less toxic and less expensive, (Floor, M., L.P.M. Hofsteede, W.P.T., Groenland, L.A. Th.Verhaar, A.P.G. Kieboom, H. van Bekkum: Preparation and calcium complexation of oxidized polysaccharides. II: Hydrogen peroxide as co-reactant in the

chlorite oxidation of dialdehyde glucans. Recl. Trav. Chim. Pays-Bas. (1989), 108, 384-392) reports that this method gives higher yields of the dicarboxy polysaccharide with superior calcium sequestering properties as compared to the reactions using chlorite as the scavenger.

[0049] By controlling the amount, nature, and conditions of oxidation or hydrolysis, the percent carboxyl groups incorporated, the position of attachment, and the molecular weight can be controlled. By effectively controlling the first periodate oxidation step, copolymers can be formed that contain both the structure of the glucose ring and the flexibility of the open ring structure with -COOH groups on them (structure IV), as shown in Figure 3.

[0050] A completely flexible copolymer structure can be engineered by partial oxidation of the -CHO groups to -COOH and reducing the remaining aldehyde groups to -OH using sodium borohydride.

[0051] The dicarboxy polysaccharides are stable at the alkaline pH of the washing process but are degraded under the acidic wastewater (pH 4-5) conditions due to their polyacetal structure. The resulting mono- and oligomeric fragments are readily biodegradable but will not form the structure needed for this application. Floor (Floor, M., L.P.M. Hofsteede, W.P.T., Groenland, L.A. Th.Verhaar, A.P.G. Kieboom, H. van Bekkum: Preparation and calcium complexation of oxidized polysaccharides. II: Hydrogen peroxide as co-reactant in the chlorite oxidation of dialdehyde glucans. Recl. Trav. Chim. Pays-Bas. (1989), 108, 384-392) shows that at a pH = 3 the dicarboxy starch can degrade up to 80% in 24 hours, while at a

pH = 7 it will only degrade 20% over a 24 hour period. This is important to note since ophthalmic solutions are usually formulated around pH=7.4. Also, this shows that the modified starch can be easily hydrolyzed. Erythronic and glyoxylic acids are the principal acidic hydrolysis fragments with minor amounts of glycolic, oxalic and formic acids. This indicates the C2-C3 dicarboxy polysaccharide structure stays intact (Floor, M., L.P.M. Hofsteede, W.P.T., Groenland, L.A. Th.Verhaar, A.P.G. Kieboom, H. van Bekkum: Preparation and calcium complexation of oxidized polysaccharides. II: Hydrogen peroxide as co-reactant in the chlorite oxidation of dialdehyde glucans. Recl. Trav. Chim. Pays-Bas. (1989), 108, 384-392).

[0052] These carboxylated cellulosic derivatives can form gels with the addition of divalent cations, such as Ca^{+2}. The rate of gelation, the gel strength and the release profile are controlled by percent carboxyl group engineered onto the polymer chain, its position on the polymer chain, and the molecular weight of the polymer chain. Floor (Floor, M., L.P.M. Hofsteede, W.P.T., Groenland, L.A. Th.Verhaar, A.P.G. Kieboom, H. van Bekkum: Preparation and calcium complexation of oxidized polysaccharides. II: Hydrogen peroxide as co-reactant in the chlorite oxidation of dialdehyde glucans. Recl. Trav. Chim. Pays-Bas. (1989), 108, 384-392) has also shown that the calcium complexing properties does not differ with respect to the type of glycosidic bond (i.e. the β-1-4 linkages of cellulose compared to the α 1-4 linkages of starches). It is also important to note that the calcium complexing ability is strongly dependent on the molecular

weight in the region M_w 10^4 to 10^5 and at least an M_w of 10^5 is required for superior calcium complexation.

[0053] The dialdehyde reaction can lead into other formations including a hydrated aldehyde, a hemiacetal, or a hemiadol (Fan, Q.C., D. Lewis et al., Journal of Applied Polymer Science **82**:1195-1202 (2001)). The structures of these are shown in Figure 5.

[0054] The periodate reaction is light sensitive and, therefore, care was taken to exclude light. While, some authors (Besemer, A.C., A.E.J. deNooy et al., Abstracts of Papers of the American Chemical Society **212** 21-Cell (1996); deNooy, A.E.J., A.C. Besemer et al., Zuckerindustrie **122**(2):126-127 (1997); Kim, U.J., S. Kuga et al., Biomacromolecules **1**(3):488-492 (2000)) suggest running the reaction at room temperature or colder, (Narayan, R., Conversion of Cellulose and Xylan into Glycols. 1983, Laboratory of Renewable Resources Engineering, Purdue University, NSF Final Report) reported the reaction could be run at slightly elevated temperatures with little interference from side reactions. For the following Examples, the periodate reaction was run at 40°C. Concentrations were used that were similar to earlier work by the prior art.

[0055] It was first proposed (Narayan, R., Conversion of Cellulose and Xylan. into Glycols, 1983, Laboratory of Renewable Resources Engineering, Purdue University, NSF Final Report) that the periodate oxidation of cellulose follow the rate law:

$$r = -\frac{d[P]}{dt} = \frac{K_1[P][C]}{K^{-1} + [P]}$$

This rate law was explained by being consistent with a mechanism involving the formation of an intermediate cellulose-periodate complex, most likely a cellulose-periodate cyclic diester which would then slowly decompose to the final products.

[0056] Later an improved explanation of the starch oxidation by periodate was proposed. It has been suggested that the kinetecs follow a 2nd order dependence at t=0, then change to another model at approximately t=10 minutes (Veelaert, S., D. Dewit et al., Polymer **35**(23):5091-5097 (1994)). This work was conducted using granular potato starch and HPLC for analysis, an improvement method over previous papers which used titration to analyze the dialdehyde formed.

[0057] Veelaert et al propose that after 5 to 10 minutes the reaction deviates from second order kinetics because of the polymeric structure of the material and the possibility of hemiacetal or acetal formation. The following two rate laws are defined for free and inhibited anhydroglucose units (an acetal neighbor):

$$\frac{d[X]}{dt} = k_1 \mu^2 [S_o]([P_o]-[X])$$

$$\frac{d[X]}{dt} = k_2 \mu(1-\mu)[S_o]([P_o]-[X])$$

Where [X]=the erythritol concentration at any time

$[S_o]$ = the initial starch concentration expressed as total initial anhydroglucose units

$[P_o]$ = initial periodate concentration

μ = 1-degree of oxidation (1-X/g)

[0058] These two equations are combined and from experimental data they observed that k_2 was much smaller than k_1. The previous formulas then can be simplified into:

$$\frac{d[X]}{dt} = \frac{k_1}{[S_o]}([S_o]-[X])^2([P_o]-[X])$$

Analytical Methods

FTIR

[0059] A PERKINS ELMER SYSTEM 2000FTIR was used to characterize samples. The samples were pressed in KBR pellets and run for 16 scans. The wavelength range was 4000 cm^{-1} to 400 cm^{-1}.

Titration

[0060] Sodium hydroxide was used to titrate against the COOH groups. The sodium hydroxide was standardized against potassium acid phthalate to obtain its normality. It was titrated to an endpoint indicated by phenolphthalein. A

concentration of approximately 1-5wt % was used. Because of the viscous nature of the material, the indicator didn't react very quickly a false endpoint would show up. The protocol used was if the indicator stayed pink (acid) for 15 minutes without lightening it was considered the endpoint.

ESEM

[0061] An environmental scanning electron microscope was used to characterize the structure of the material. The instrument is an ELECTROSCAN 2020 environmental scanning electron microscope. For these samples, there was a beam voltage of 25 kV with an emission current of 49 uA. The water pressure was varied from 2 Torr to 9 Torr.

Dicarboxy Matrix Synthesis and Characterization Oxidation Methods:

[0062] First the method of oxidation was examined.
The following three methods were used with the native starch.

[0063] *Method I*

Table 4. Explanation of Oxidation Methods

		Reaction Time	Results
Method 1	1-step oxidation with sodium hypochlorite	24 hours	Completely water soluble product that is extremely hydroscopic in the presence of air. Also yellows when exposed to air.
Method 2	1-step oxidation with ozone	6 hours	Non-water soluble product that shows very little carboxyl peaks in IR.
Method 3a	2-step oxidation with sodium m-periodate followed by sodium chlorite	6 hours + 12-24 hours	Gummy product that is soluble in water. Swells quickly when rewetted.
Method 3b	Same as above, except that special care was taken to keep the dialdehyde from drying out in between reactions	3 hours + 6 hours	Gummy product that is soluble in water Swells quickly when rewetted

[0064] From the FTIR in Figure 6, the carbonyl stretch around 1740 cm^{-1} shows that the different methods had different impacts on oxidation. While not quantitative, its comparison can be made by comparing it to the neighboring 1620 cm^{-1} (C-OH) peak. The ozonated starch, so a very slight shoulder around 1740 cm^{-1} indicating that there was some reaction. The hypochlorite method and Method 3a show that there is slightly more carbonyl present but the peak is much smaller than the 1620 cm^{-1}. These lead to the possibility that the water solubility of the material may be due to hydrolysis of the starch as opposed to too high a carboxyl presence. As seen in the top peak, there is a high level of carboxyl and the peak is stronger than the 1620 cm^{-1} peak. The difference between

the 3a and the 3b method, which in this case had the exact same reactant concentrations, indicates that the structure of the dialdehyde product before the second oxidation plays a very important role in the subsequent oxidation. While this example did not indicate some of the reactions so a presence of an aldol reaction. As seen in Figure 7, the additional peak at 1784 cm^{-1} indicates the presence of an anhydride which could indicate the presence of the hemi-aldol structure. Specifically a strong anhydride of the structure R-COOCO-R shows a carbonyl stretch at 1790-1740 cm^{-1}. This would be consistent of the oxidation of the hemi-aldol structure.

Polysaccharide Choice

[0065] Oxidation Method 3a was tried on different saccharides including native corn starch, waxy starch, cellulose, pretreated cellulose, xylans and glucose. The native and waxy starch produced the best results. The cellulose produced similar results but the reaction time was longer and required pretreatment with a strong acid. Because of that the starch was used in subsequent reactions. The following chart summarizes the results of the products.

Table 5. The results of the oxidation of different saccharides.

Material	Comments	Periodate Oxidation Reaction time	Chlorite Oxidation Reaction Time	Results
Native Starch		6 hours		Good results, high dicarboxy content, material swells
Waxy starch	Waxy pearl 1108	6 hours		Good results, high dicarboxy content, material swells
Cellulose	Sigmacell from Sigma-Aldrich	24 hours		Only small percentage was oxidized
Pretreated Cellulose	Sigmcell pretreated with phosphoric acid and sodium hydroxide	24 hours		Good results, high dicarboxy content, material swells
Glucose		24 hours		Material was over oxidized
Xylans		7 hours		No change in the material

Titration

[0066] Titration with sodium hydroxide was used to measure the amount of carboxyl groups present in the samples. All of the values reported are in terms of carboxyl groups/anhydrogluco ring. For example 100% would indicate that every anhydrogluco ring has one carboxyl group present. Theoretically, the maximum value would be 300% since the C-2, C-3 and C-6 carbon could potentially contain a carboxyl group. Besides actual content, the titration also could be used to

quantify the reproducibility of the reaction.

[0067] Because of the heterogeneity of the material produced using Method 3a for oxidation, titration of those samples was not reproducible. A single sample would have values ranging from 10% - 30%. This proved that the material was not being produced in a consistent manner which further confirms that other structures such as hemi-aldols were being formed. Table 4 shows the titration results for the material produced by Method 3b. The standard deviation of titrating a sample in duplicate was from 0.01%-3.1%, which were acceptable values. Also it can be seen from the table that materials produced using the same periodate to starch ratio showed consistent carboxyl content. All of the data presented here were for reactions using 3 hours for the periodate reaction followed by 6 hours for the chlorus acid oxidation with waxy corn starch as the starting material. Figure 8 graphically shows the relationship between the periodate ratio used and the resulting carboxyl content. A logarithmic dependence can be explained by the fact that as more dialdehyde is the polysaccharide becomes more susceptible to acid hydrolysis breaking the chain into smaller molecular weight chains. These chains are removed during the washing of the material and therefore do not show up in the titration.

Table 6. Titration results

Sample	Periodate ratio %	COOH/ring	Std. Dev.
44	50	46.7%	0.01%
45	50	45.0%	0.02%
46	30	29.1%	0.03%
47	10	0.8%	0.1%
48a	30	27.8%	2.1%
48b	30	24.2%	1.8%
49a	20	15.3%	3.1%
49b	20	12.7%	-
50	50	42.7%	1.9%
51a	20	14.3%	1.5%
51b	20	13.6%	2.0%
52a	80	54.7%	1.5%
53a	100	58.2%	0.0%

[0068] This data deviates from the data presented by Veelaert (Veelaert, S., et al., Polymer **35**(23):5091-5097 (1994)), which shows a linear dependence as the stoichiometric amount is increased. This data may be explained by the fact that high amylopectin is being used. This highly branch material may be stericly hindering the oxidation as higher concentrations of periodate are used.

Periodate Oxidation Kinetics Data

[0069] Samples were taken during the periodate oxidation of starch and of cellulose at different varying time intervals. The UV spectrophotometer was used to analyze the samples since the periodate has a maximum peak at 223 nm. Data was used from previously obtained periodate data and new data and compared to the model.

[0070] A close fitting relationship was found using the Veelaert model. A Runge-Kutta differential equation solver set

up on Excel was used to solve for the rate constants. Two separate reactions, one for cellulose and one for starch were compared to the model.

[0071] As can be seen in Figures 9, 10 and 11, the models show a close relationship. The rate constants for each are using the endpoint of three hours and the carboxyl content at that point. This introduces error because the assumption is that the dialdehyde is fully oxidized to carboxyl groups.

Table 7. Calculated rate constants for the periodate oxidation of starch

Sample	%dialdehyde	K(calculated) L/mmole/min
47	10	1.500E-08
49b	20	1.400E-07
51a	20	1.250E-07
46	30	1.300E-07
48a	30	1.200E-07
44	50	3.492E-07
45	50	3.205E-07
50	50	2.800E-07
52a	80	8.300E-07
53a	100	1.350E-06

Design of Experiments

[0072] Stat-Ease software, Design-Expert 5.0 was used to create a design of experiments to see how the initial periodate ratio affected the product. This was used to optimize the reaction to predict the most desirable product. The final results of this were used as the case that was scaled up as set forth hereinafter. Acid content, overall reaction yield and dispersibility were used to qualify the product. The titration results were used for the acid

content. Because of the logarithmic relationship shown in Figures 12 to 15, the exponential values of the carboxyl content were used. The yield was calculated by looking at the percentage of the polymeric material left at the end of the reaction compared to the theoretical amount that could be produced and dispersibility was rated on a scale of 0 to 3. On this scale a 3 indicated that within 10 minutes of adding the material to water it appeared completely dispersed, a 2 indicated that in that time frame the majority of the material was swollen and dispersed, a 1 indicated that a majority of the material was not dispersed but at the least the material had swollen considerably and a 0 indicated that there was no visible hydration of the material within the 10 minute timeframe. This is an important factor to consider for manufacturing and formulating of a final product and to ensure that the drug can be uniformly distributed in the matrix. Carboxyl contents and reaction yield showed a statistically significant relationship to the periodate ratio used and the dispersibility show a relationship with a $p=0.0761$.

Response: Yield

ANOVA for Response Surface Linear Model

Analysis of variance table [Partial sum of squares]

Source	Sum of Square	DF	Mean Square	F Value	Prob>F	
Model	0.48	1	0.48	40.98	0.0002	
Significant						
A	*0.48*	*1*	*0.48*	*40.98*	*0.0002*	
Residual	0.094	8	0.012			
Lack of Fit	*0.066*	*4*	*0.016*	*2.30*	*0.2204*	*not significant*
Pure Error	*0.029*	*4*	*7.166E-003*			
Cor Total	0.58	9				

The Model F-value of 40.98 implies the model is significant. There is only a 0.02% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob >F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant.

If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Final Equation: Yield =+0.97444 -0.81743 *periodate ratio

Response: COOH %/ring

ANOVA for Response Surface Linear Model

Analysis of variance table [Partial sum of squares]

Source	Sum of Square	DF	Mean Square	F Value	Prob>F
Model	0.56	1	0.56	70.70	<0.0001
Significant					
A	*0.56*	*1*	*0.56*	*70.70*	*<0.0001*

Residual	0.063	8	7.864E-003		
Lack of Fit	*0.061*	*4*	*0.015*	*28.71*	*0.0033*
significant					
Pure Error	*2.117E-003*	*4*	*5.293E-004*		
Cor Total	0.62	9			

The Model F-value of 70.70 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob >F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Final Equation: COOH %/ring = +1.03430 + 0.87633 *periodate ratio

Response: Dispersibility

ANOVA for Response Surface Linear Model

Analysis of variance table [Partial sum of squares]

Source	Sum of Square	DF	Mean Square	F Value	Prob>F
Model	3.45	1	3.45	4.16	0.0761
Not significant					
A	*3.45*	*1*	*3.45*	*4.15*	*0.0761*
Residual	6.65	8	0.83		
Lack of Fit	*3.49*	*4*	*0.87*	*1.10*	*0.4641 not significant*
Pure Error	*3.17*	*4*	*0.79*		
Cor Total	10.10	9			

The Model F-value of 4.15 implies there is a 7.61% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob >F" less than 0.0500 indicate model terms are significant. In this case there are no significant model terms. Values greater than 0.1000 indicate the model terms are

not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Final Equation: dispersibility = +1.33978 +2.18232 *periodate ratio

Optimization Results

The following constraints were set to find the optimal periodate ratio used.

Constraints

Name	Goal	Lower Limit	Upper Limit	Importance
Periodate ratio	Is in range	0.1	1	3
Yield	Maximize	0.136	1	3
COOH %/ring	Is target=1.398	1.007	1.789	3

Solution

Periodate ratio	Yield	COOH %/ring
0.42	0.634683	1.39854

[0073] This design of experiments could be expanded in the future to incorporate the release data and calculated diffusion coefficients to develop a predictive model with reactant molarity as the input and diffusion coefficients as the output.

ESEM

[0074] As seen in the ESEM images (see Figures 16A to 16E)

there is a difference between native starch and the dicarboxy starch. It can be observed that the oxidation process destroys the granular structure of the starch (Figure 18A), releasing the amylase and amylopectin from the structure creating a smooth and flexible material (Figure 16D). The ESEM is run under vacuum so it is impossible to observe the hydrated structure. However, the swelling and subsequent dehydration of the material can be observed while the material is first wetted and the vacuum chamber comes to equilibrium (Figure 16E).

Kinetic considerations and scale up

[0075] In Figure 17, a large-scale batch process for the production of the dicarboxy starch is described.

Formulated product required/annually	200000 kg
Assume 10,000,000 bottles & 20 ml each	
Amount of dicarboxy starch needed/annually	2000 kg
# of batch runs annually	50
Amount/barch needed for 42% dialdehyde	40 kg
Yield	63.47%
Reactants/batch	
Starch	60.9 kg
Sodium m-periodate	33.8 kg
Water	6282.4 kg
Acetic Acid	13.6 kg
Hydrogen Peroxide	45.1 kg
Na-EDTA	0.8 kg
Sodium Chlorite	42.5 kg

[0076] Reactor 1 is a 500 gallon stainless steel jacketed reactor. Water would be used to heat the reactor to 40°C.

The second reactor is a stainless steel 1250 gallon reactor. Chlorine byproduct is controlled and quenched accordingly. The ethanol/water washwater is recycled using a basic distillation column.

[0077] A suitable filter that ionically repels the material or at least, not attract it. The iodate can be reoxidized to paraperiodate using sodium hypochlorite which then will release the metaperiodate ion.

[0078] Reduction of the aldehyde groups to -OH groups provides hydroxyl moieties in addition to carboxylic moieties.

In vitro UV-Vis spec

[0079] The drug release profiles were conducted using two different set-ups as more equipment became available.

Stir plate method

[0080] In the first set up in Figure 27, a 15 ml polystyrene centrifuge tube was modified by cutting the tip off and placing a dialysis membrane (Sigma) with a molecular cut-off of 12,000 over the open end. The membrane was secured by wrapping Teflon® taping tightly around the tube. The diffusion surface with this set up was 15 mm and 5 ml of the formulated drug was placed in the tube. Twenty-five milliliters of release medium was placed in a polyethylene cup which was modified by cutting a hole the diameter of the tube in the top along with another hole for sampling and temperature measurements. A 1" stirbar was placed in this cup and the cup was placed in a water bath kept constant at 37°C on a stir plate. One milliliter samples were taken at time varying intervals and 1 ml of fresh release medium was added

to keep the volume constant at 25 ml. The composition of the release medium, simulated tear solution was as follows:

Simulated Tear Solution I

Sodium Chloride	0.67 g
Sodium bicarbonate	0.2 g
Calcium chloride dehydrate	0.008 g
Water	to 100 g

[0081] This method led to variability because of the variations in the rpm of the stirbar between stir plates. The schematic system is shown in Figure 27.

USP dissolution method

[0082] The second method in Figure 28 for obtaining release profiles was using a Hanson EZ-lift dissolution system which had 6 separate chambers that were all kept in the same constant temperature bath which was regulated by a feedback loop. Each chamber had a rotating paddle attached to the same drive motor. One liter beakers were used to hold the release medium and they were filled with a specified amount ranging from 400 ml-650 ml. The formulated drug was placed in a well 5 ml with a diameter of 5 cm which was covered with the same dialysis membrane. Again, 1 ml samples were taken at varying intervals; however, the release medium was not replaced in these experiments since the volume difference was considered negligible. A schematic of the system is shown in Figure 14.

[0083] A Perkins-Elmer Lambda 900 Ultraviolet-Visible Spectrophotometer was used to determine the concentration of the drug in the drug release profiles. The strongest peak was at 290 mm and the absorbance there was used to determine the

concentration. The UV/VIS integration time was 0.3600 s, and the slit width was set to 2.00 nm. Deionized water was used as the reference, since the tear solution did not contribute to the peak at 290 nm. The software used to obtain the data was UV Winlab for Lambda 900, version 2.90.02.

Drug Release Profiles

[0084] Ofloxacin in Figure 18 is an antibacterial agent belonging to the fluoroquinolone family with molecular weight of 361.37. Of the available fluoroquinolones, ofloxacin is one of only usually given as a single agent and has been shown to have the best aqueous humor penetration. As an ophthalmic formulation, ofloxacin is formulated as a 0.3% w/v solution and goes by the trade name OCUFLOX. According to Allergan's prescribing information packet, OCUFLOX solution is unbuffered and formulated with a pH of 6.4 (range - 6.0 to 6.8). It has an osmolality of 300 mOsm/kg. Ofloxacin is a fluorinated 4-quinolone which differs from other fluorinated 4-quinolones in that there is a six member pyridobenzoxazine ring from positions 1 to 8 of the basic ring structure.

Drug Release Results

[0085] The drug release profiles were studied using a Perkin Elmer Lambda 900 ultraviolet-visible spectrophotometer. The absorbance spectrum for the drug ofloxacin can be seen in Figure 19. The strongest peak at 290 nm was used to determine concentration of ofloxacin as compared to a calibration curve (Figure 20). The absorbance at concentrations of 0.0036% w/v to 0.00075% w/v was found to be linearly dependent and measurable using the parameters described in the analytical

technique chapter. Some of the samples had to be diluted to 1 part sample to 2 parts plain tear solution to have samples in a measurable range. A concentration method was developed to read the output only the absorbance at 290 nm. This eliminated the need of developing full spectra for all of the samples. The calibration confirmed that absorbance was linear with concentration from a range of absorbance from 0-2.5.

[0086] The drug release profiles were conducted using the apparatus of Figures 27 and 28 and are referred to as the stirplate method and the USP transdermal method.

Stirplate method results

[0087] Initially the release of the dicarboxy starch was compared to that of GELRITE to see if it exhibited similar release properties. As seen in Figure 21 to 24, over a period of one hour the dicarboxy starch and the GELRITE released the ofloxacin in a similar manner.

Results from the USP method

[0088] Figure 25 shows the release profiles from three (3) different dicarboxy starches (20% dicarboxy, 50% dicarboxy and 80% dicarboxy) compared to the release profile of GELRITE. All were formulated using 1 wt% of the matrix in a phosphate buffer, pH = 7.4. There is no statistical difference between the release profiles of the materials with different carboxy concentrations.

Modeling the Diffusion Method

[0089] The data obtained from the USP dissolution method was used compared to the models predicted by the Higuchi Model. As indicated by the model, the release of the drug

from a swollen hydrogel is diffusion controlled and should follow a square root time dependence when the percentage released is less than 60%. As seen in Figure 26, the drug release is consistent with that model because all experimental data for each of the matrices can be fitted with a linear fit with an R^2 value greater than 0.97. The varying slopes of the lines indicate that there are different apparent diffusion coefficients for each of the different materials. This confirms the fact that material can be engineered to change the release profiles. The release profile with no matrix appears linear when plotted against the square root of time, however, there is a higher linear correlation when it is plotted against time which is consistent with standard diffusion through a membrane (Saltzman, M., Drug Delivery:Engineering Principles for Drug Therapy. New York, Oxford University Press (2001)).

[0090] Following the Higuchi model for the same material, surface area and volume, the diffusion coefficient should be able to be calculated by changing the concentration of the drug. To calculate this, a formula with 1% of the 20% dicarboxy starch was made up with varying concentrations of ofloxacin. These runs were conducted once each. The percent released was plotted against the square root of time and the slopes of the lines were found. According to Higuchi model, when rearranged for the dimensionless percent released, the slope of the line, y, should be equal to:

$$Y=2A(D_m/\Pi)^{0.5}$$

[0091] Following this equation the slope of the lines showed were equal for the same matrix and release area. As

seen in Figure 26, the slopes vary for each of the release profiles. Since each of these runs were conducted only once, the difference could be due to the number of runs. The 0.3% formulation may becoming close to the solubility limit of the drug in which case the Higuchi model presented would deviate. If the values for the 0.3% value are removed, the average of the slope of the lines becomes 0.0665±95 which is considered a reasonable deviation. Using that number, an apparent diffusion coefficient can be calculated with the answer being 0.000225 cm^2/s.

[0092] In conclusion, it appears that the release profiles can be modeled using the Higuchi equation. Monitoring the ofloxacin concentration with the UV-visible spectrophotometer provides a good means to analyze the release profile.

[0093] It is intended that the foregoing description be only illustrative of the present invention and that the present invention be limited only by the hereinafter appended claims.

WE CLAIM:

1. A method for providing a topical timed release of a medicament for the eyeball of an animal in need thereof which comprises:

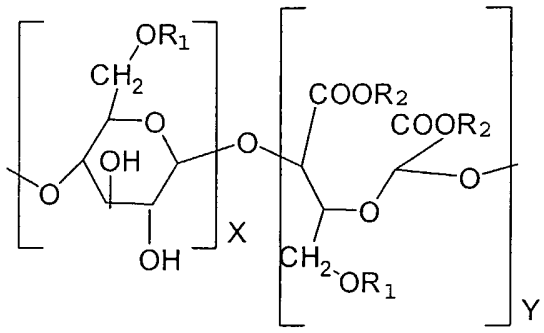
(a) providing a composition which comprises: a medicament; and a chemically modified polysaccharide (CMP) comprising linked saccharide rings with ring opened saccharide units at C_2 and C_3 bond and containing carboxylic acid moieties or water dispersible salt as a random copolymer wherein the CMP is water dispersible to form a clear solution as a time release adjuvant for the medicament; and

(b) topically providing the medicament on the eyeball to provide the timed release.

2. The method of Claim 1 wherein in the CMP all or portions of C_2 and C_3 is a hydroxyl group other than at units which have carboxylic acid group.

3. The method of Claim 1 wherein in the CMP both C_2 and C_3 are carboxylic acid groups.

4. The method of Claim 1 wherein the CMP is a copolymer of linked units of the formula:



wherein ring opened units in the copolymer are between 10 to 90 mole percent, wherein R_1 is H or COOR where R is alkyl or aryl, and wherein R_2 is H, alkyl or an aryl group containing 1 to 12 carbon atoms.

5. The method of Claim 1 wherein the animal is a mammal.

6. The method of Claim 5 wherein the mammal is human.

7. A pharmaceutical composition for topical treatment of the eyeball which comprises:

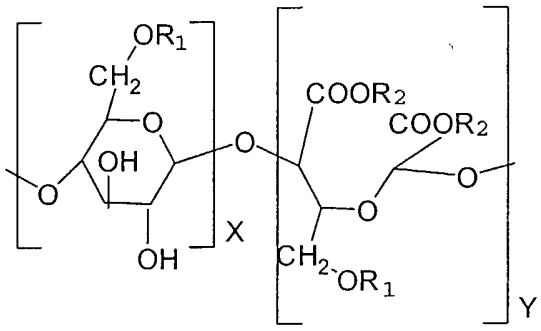
(a) a medicament for the eyeball; and

(b) a chemically modified polysaccharide (CMP) comprising linked saccharide rings with ring opened saccharide units at C_2 and C_3 bond and containing carboxylic acid moieties or water dispersible salt as a random copolymer wherein the CMP is water dispersible to form a clear solution as a time release adjuvant for the medicament onto the eyeball.

8. The composition of Claim 7 wherein in the CMP one or both of C_2 and C_3 is a hydroxyl group other than at units which have carboxyl group.

9. The composition of Claim 7 wherein in the CMP both C_2 and C_3 are carboxylic acid groups.

10. The composition of Claim 7 wherein the CMP is a copolymer of linked units of the formula:



wherein ring opened units in the copolymer are between 10 to 90 mole percent, wherein R_1 is H or COOR where R is alkyl or aryl, and wherein R_2 is H, alkyl or an aryl group containing 1 to 12 carbon atoms.

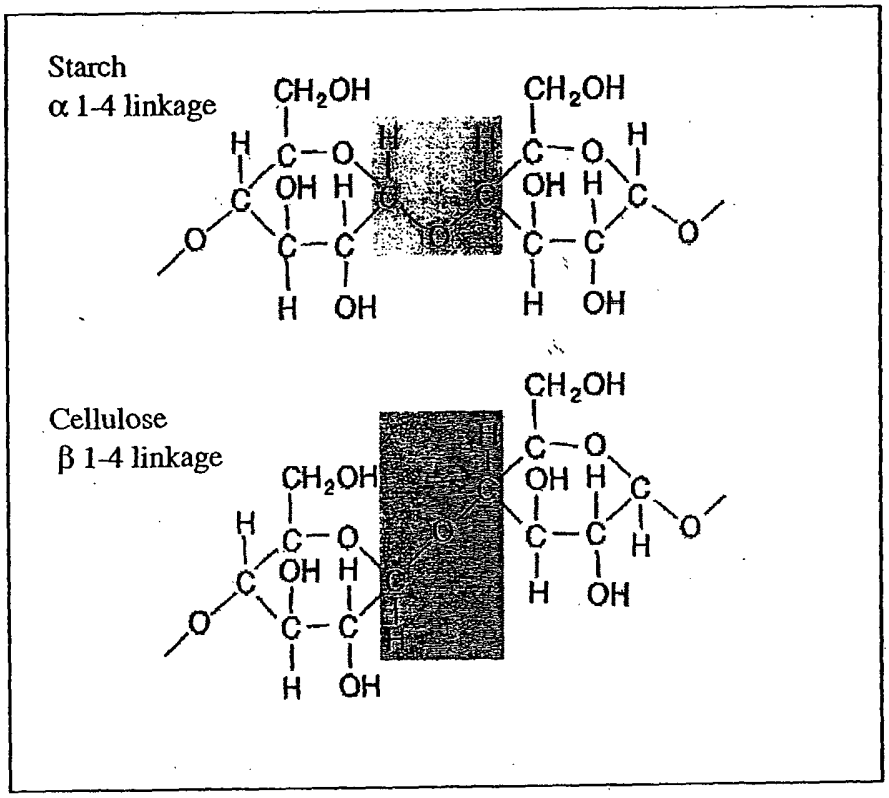


FIGURE 1

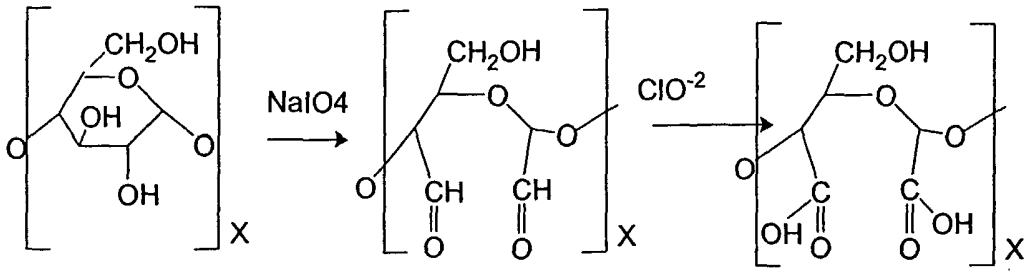


FIGURE 2

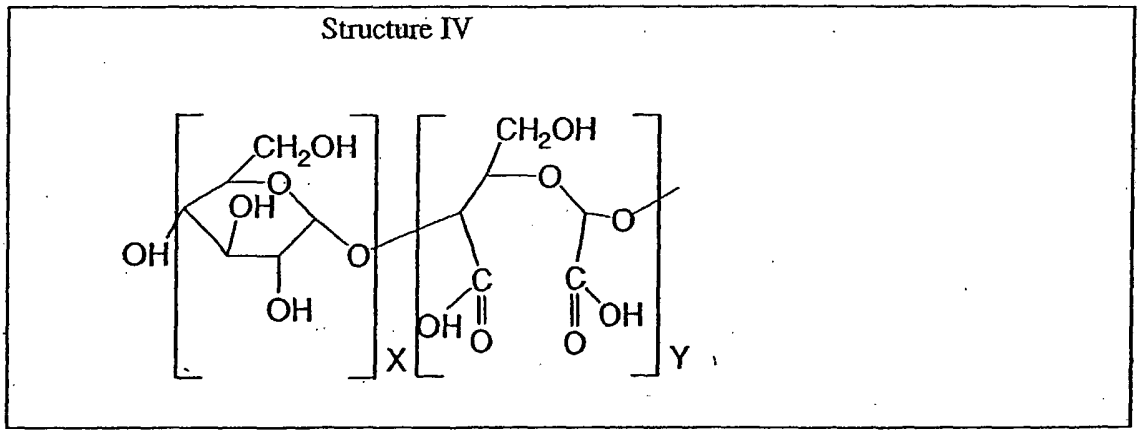


FIGURE 3

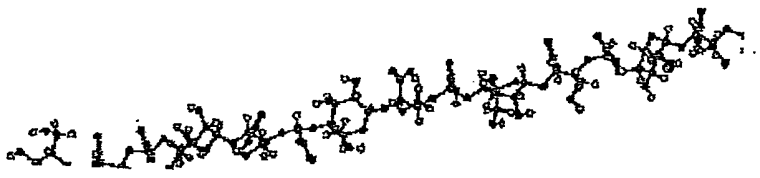


FIGURE 4

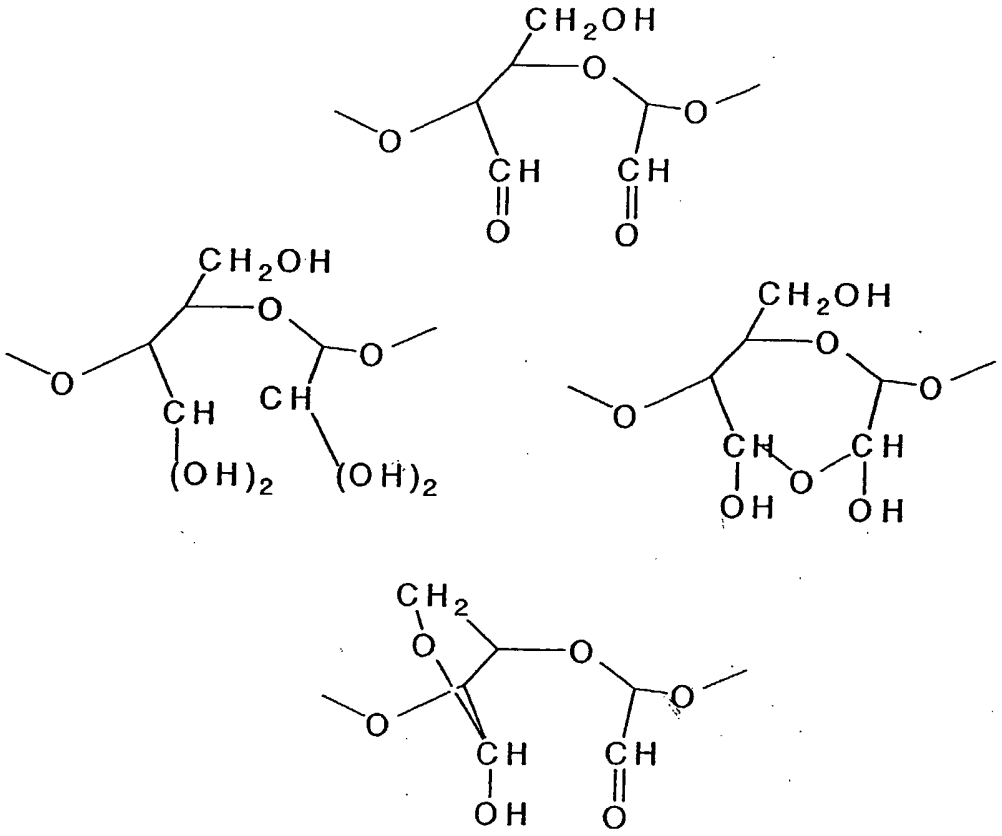


FIGURE 5

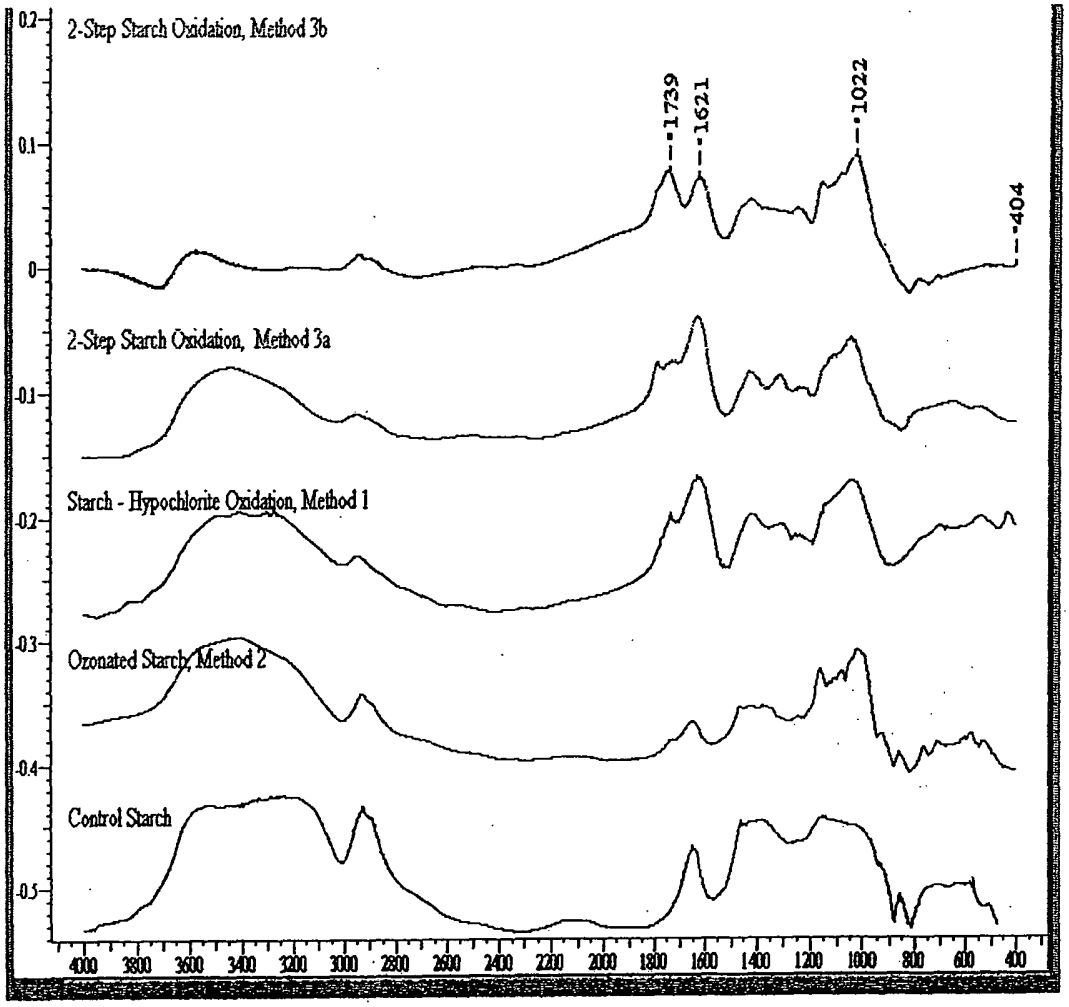

2-Step Starch Oxidation, Method 3b
1739
1621
1022
404
2-Step Starch Oxidation, Method 3a
Starch - Hypochlorite Oxidation, Method 1
Ozonated Starch, Method 2
Control Starch
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
4000 3800 3600 3400 3200 3000 2800 2600 2400 2200 2000 1800 1600 1400 1200 1000 800 600 400

FIGURE 6

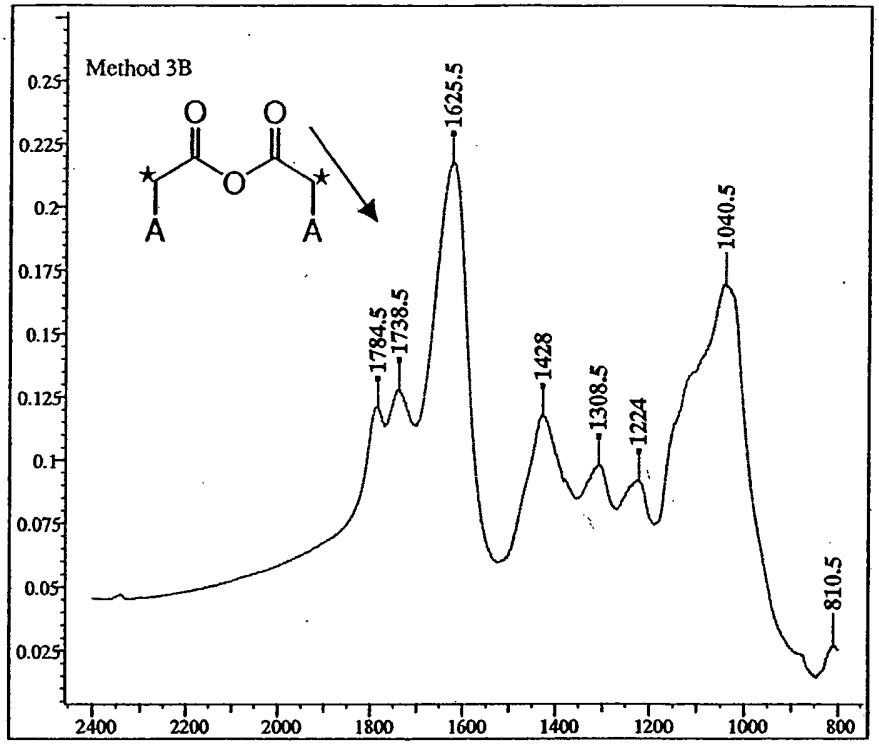

Method 3B
O
O
O
A
A
1784.5
1738.5
1625.5
1428
1308.5
1224
1040.5
810.5
0.25
0.225
0.2
0.175
0.15
0.125
0.1
0.075
0.05
0.025
2400
2200
2000
1800
1600
1400
1200
1000
800

FIGURE 7

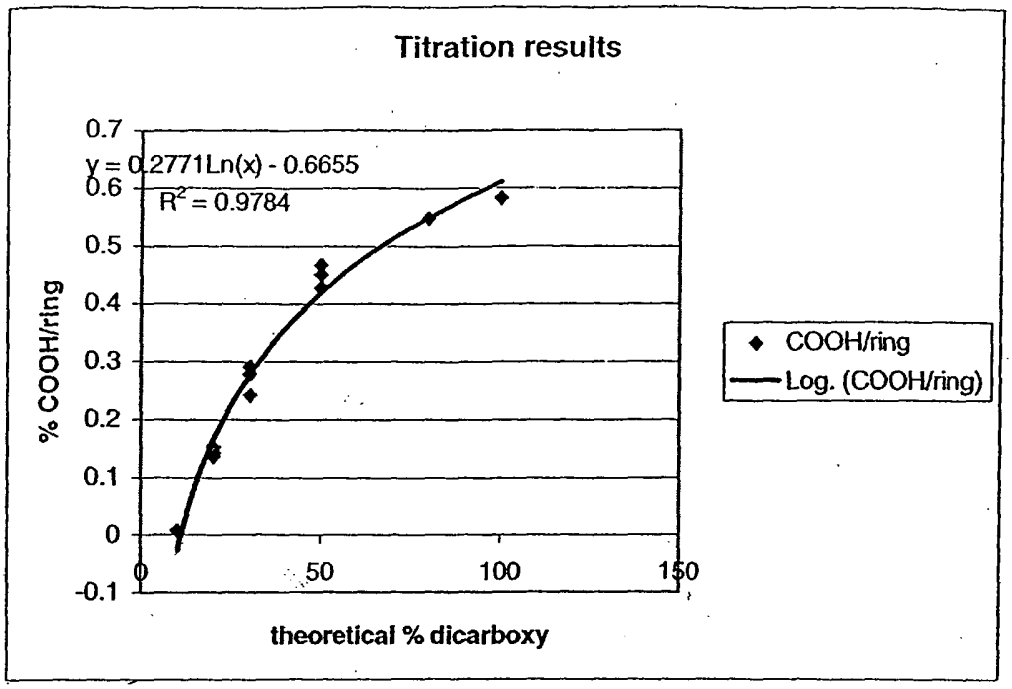


FIGURE 8

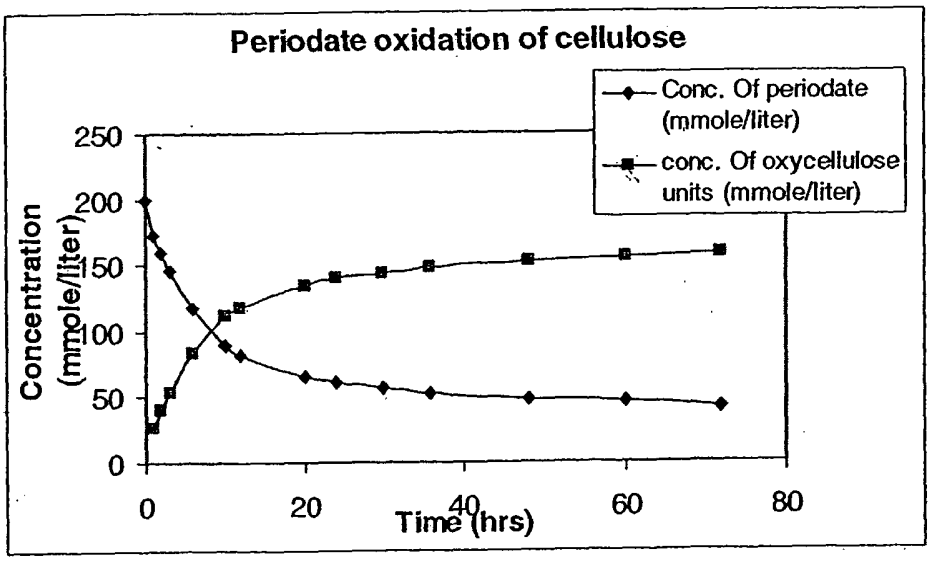


FIGURE 9

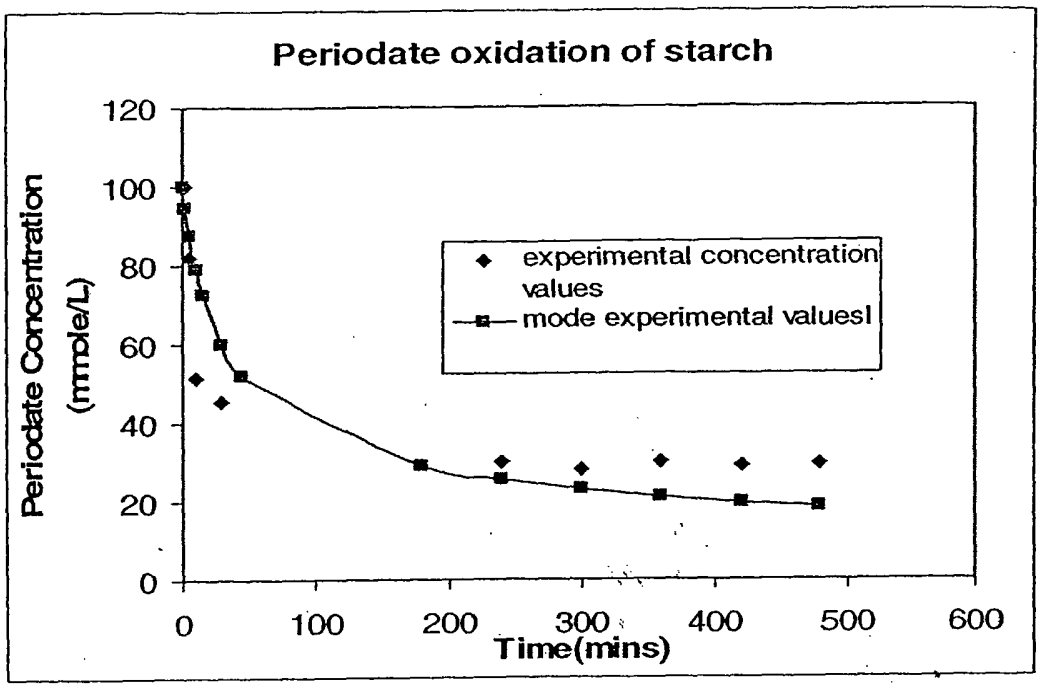


FIGURE 10

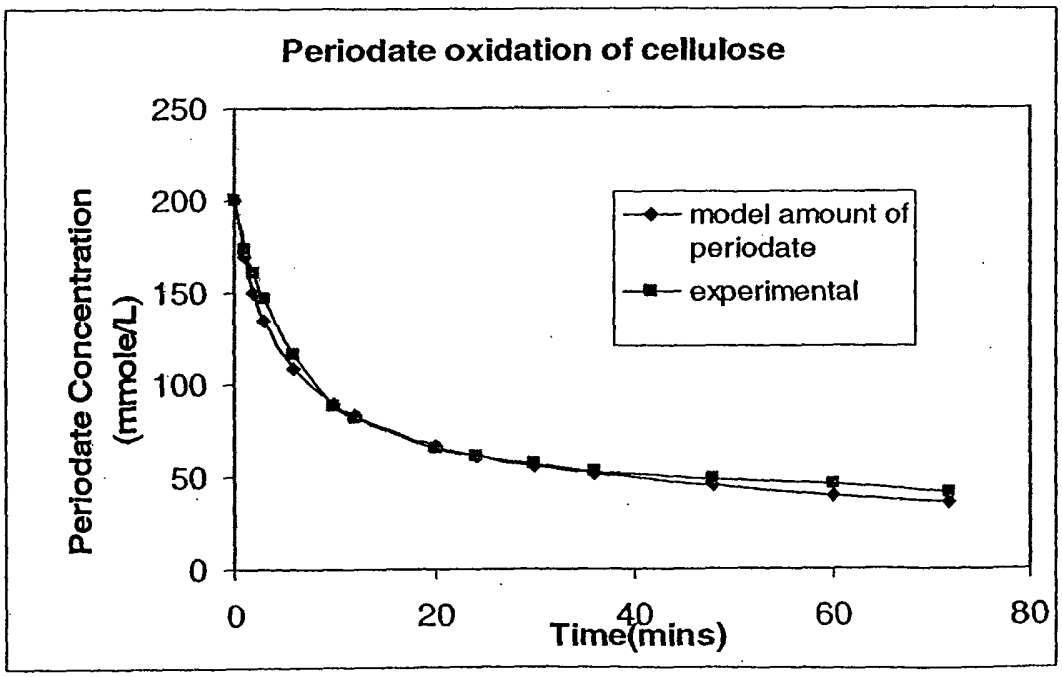


FIGURE 11

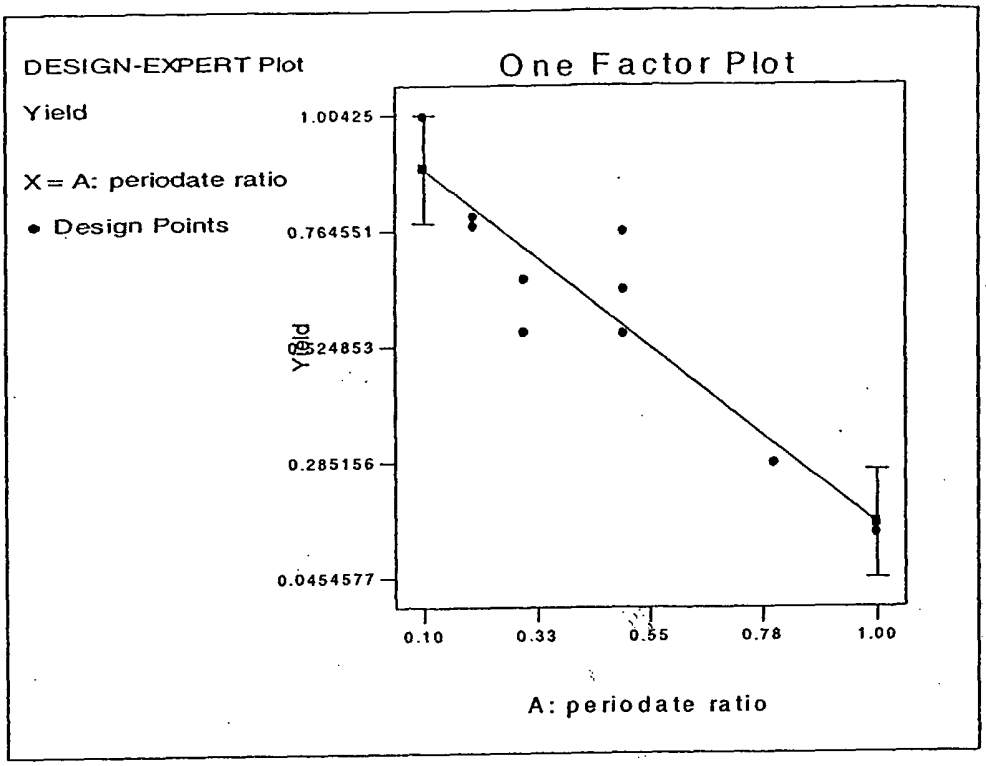


FIGURE 12

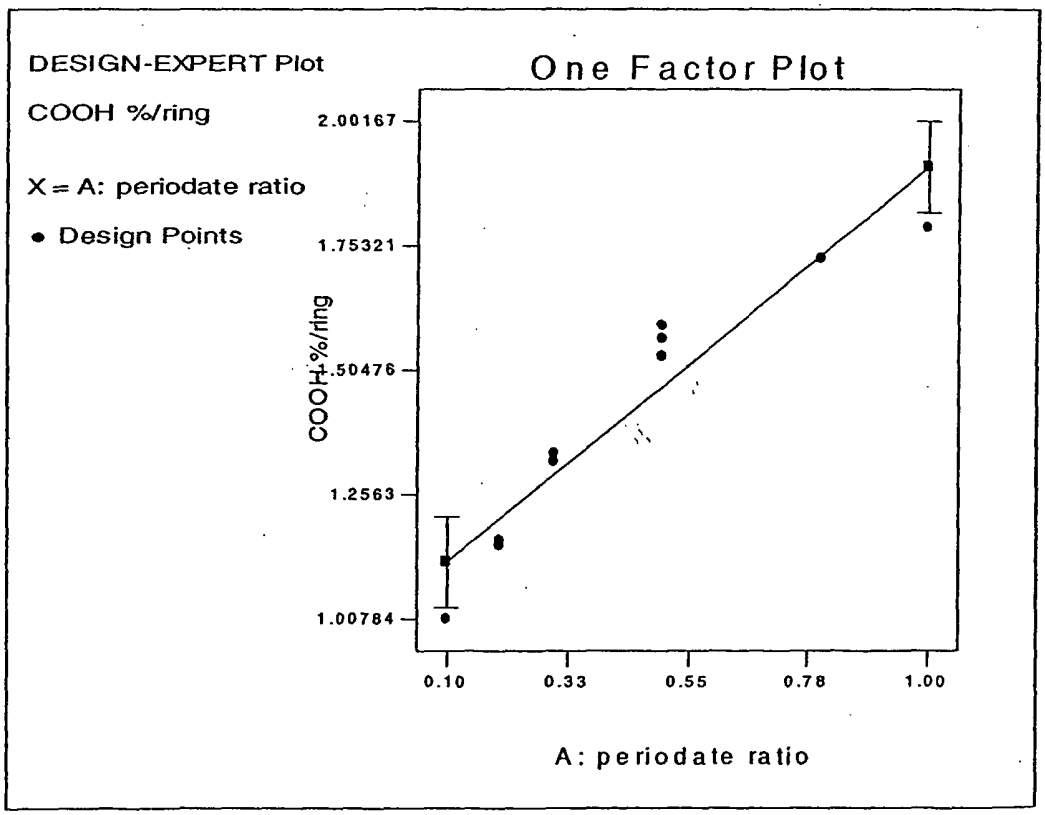


FIGURE 13

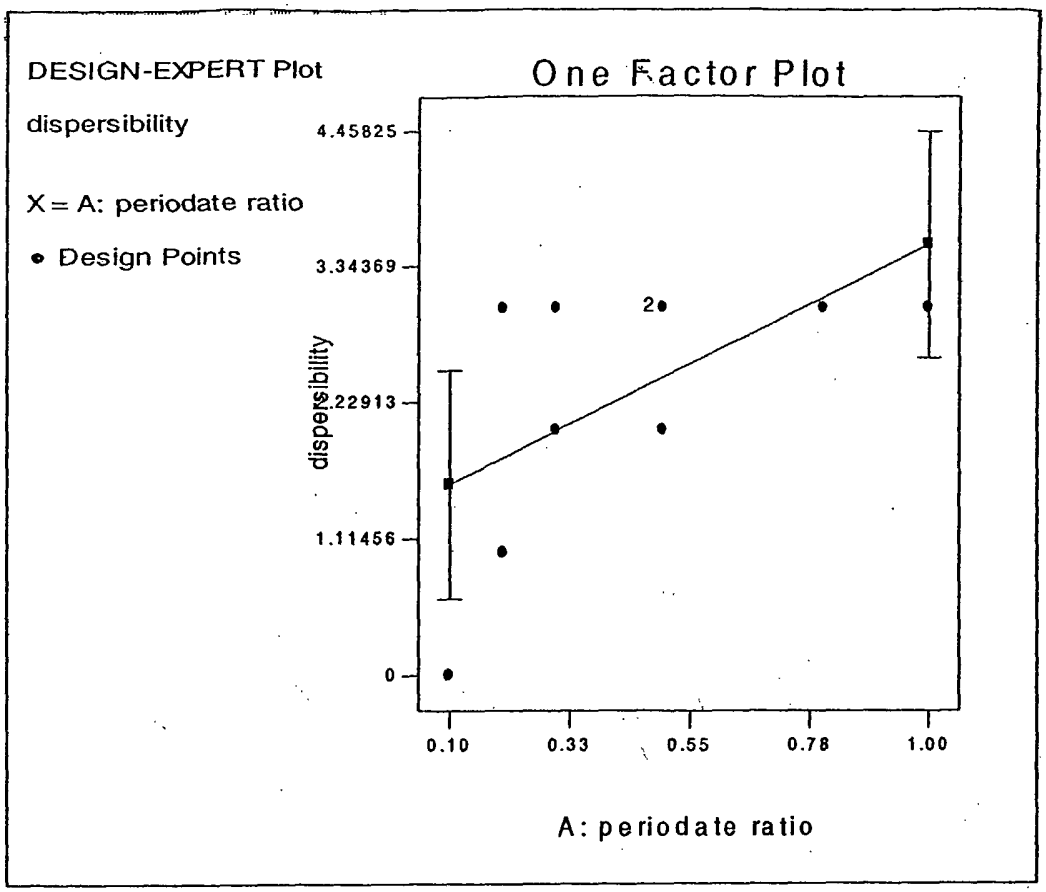

DESIGN-EXPERT Plot
dispersibility
X = A: periodate ratio
Design Points
One Factor Plot
4.45825
3.34369
2.22913
1.11456
0
dispersibility
2
0.10
0.33
0.55
0.78
1.00
A: periodate ratio

FIGURE 14

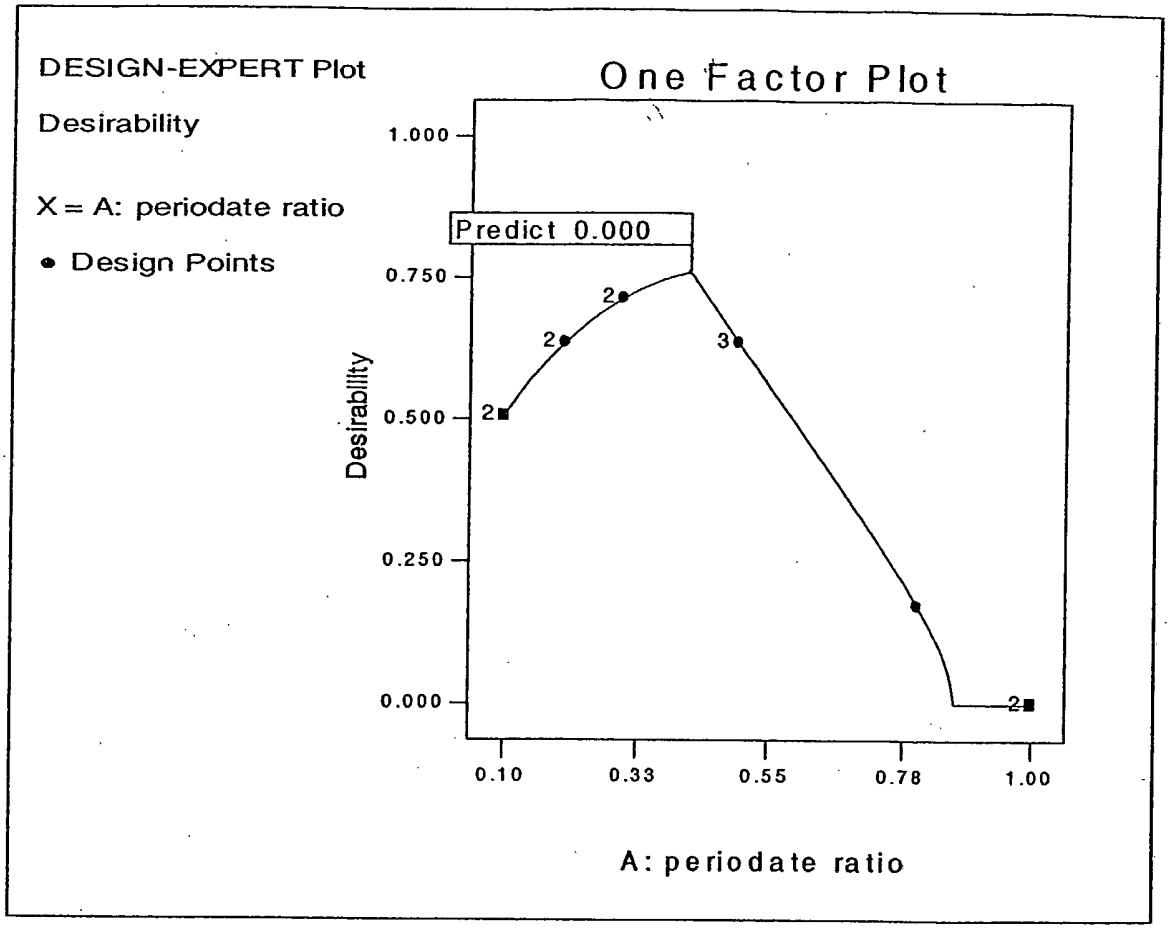

DESIGN-EXPERT Plot
Desirability
X = A: periodate ratio
Design Points
One Factor Plot
Predict 0.000
Desirability
1.000
0.750
0.500
0.250
0.000
0.10
0.33
0.55
0.78
1.00
A: periodate ratio

FIGURE 15

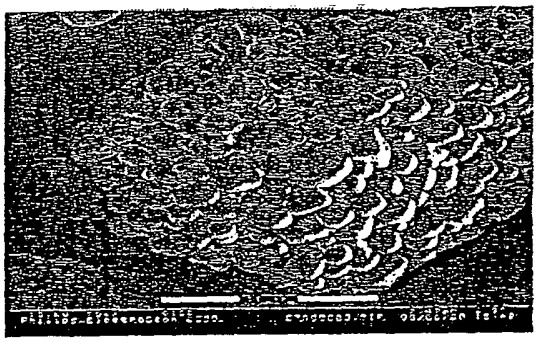


FIGURE 16A

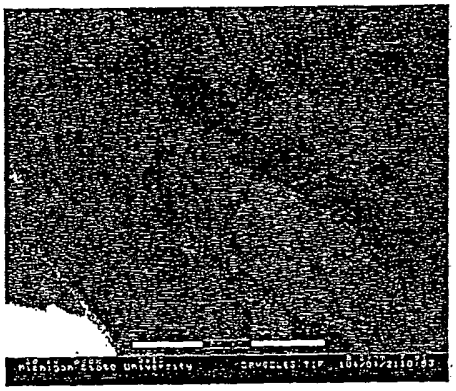


FIGURE 16B

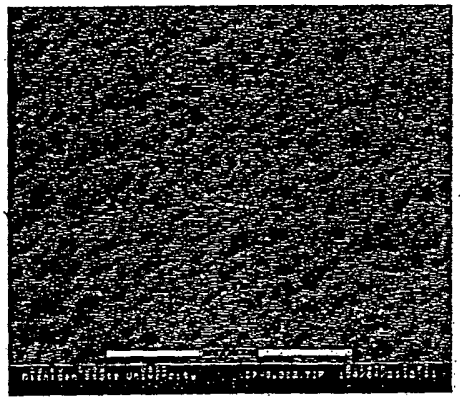


FIGURE 16D

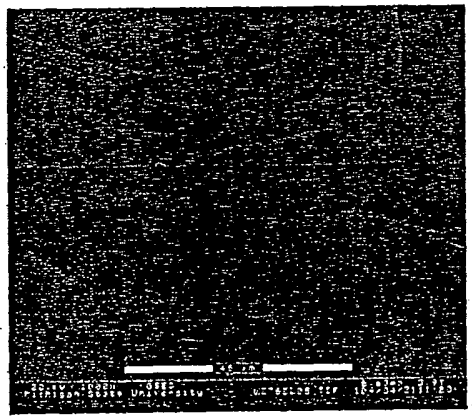


FIGURE 16C

FIGURE 16E

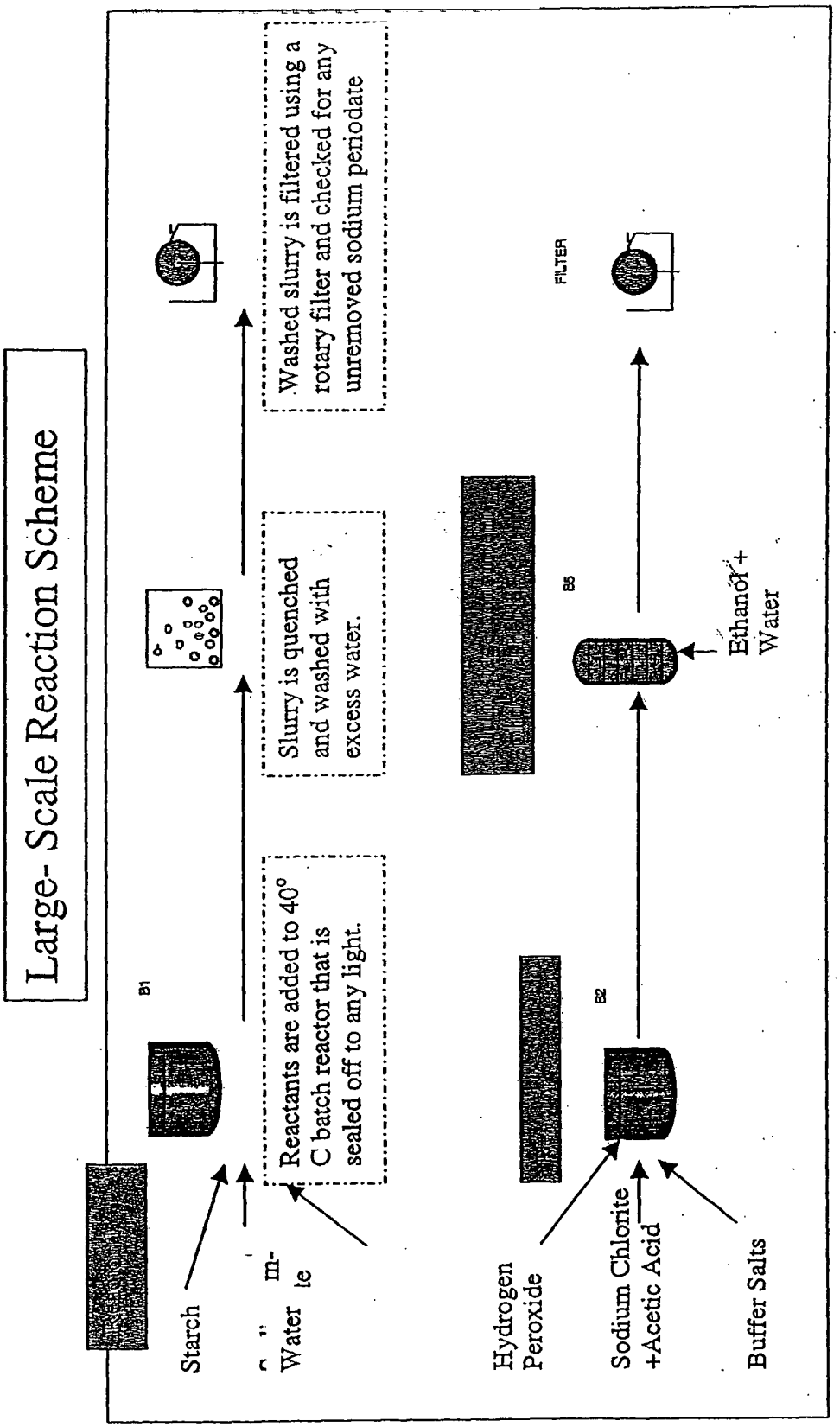


Figure 40. Large-scale reaction scheme

FIGURE 17

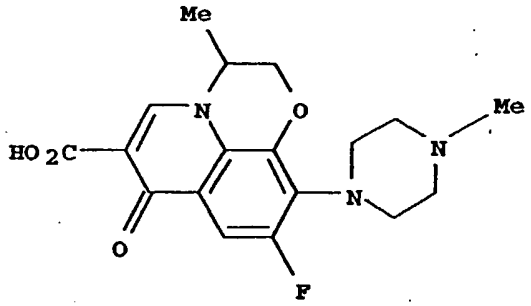


FIGURE 18

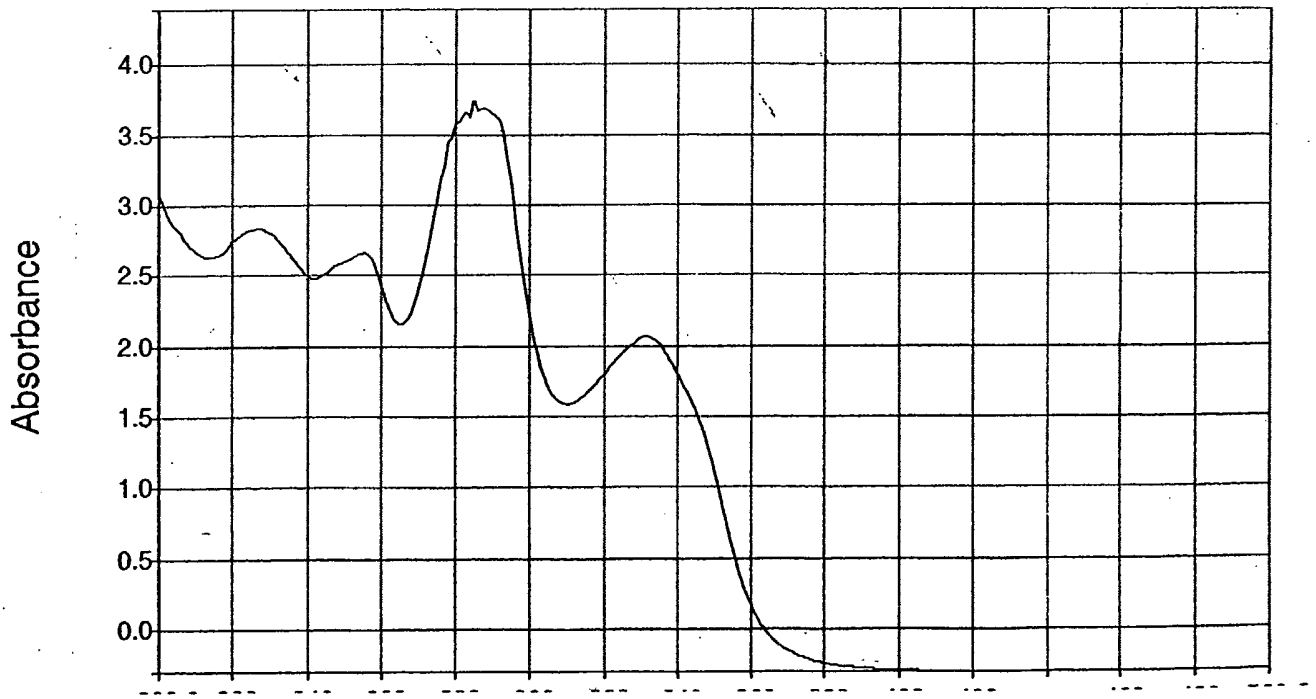


FIGURE 19

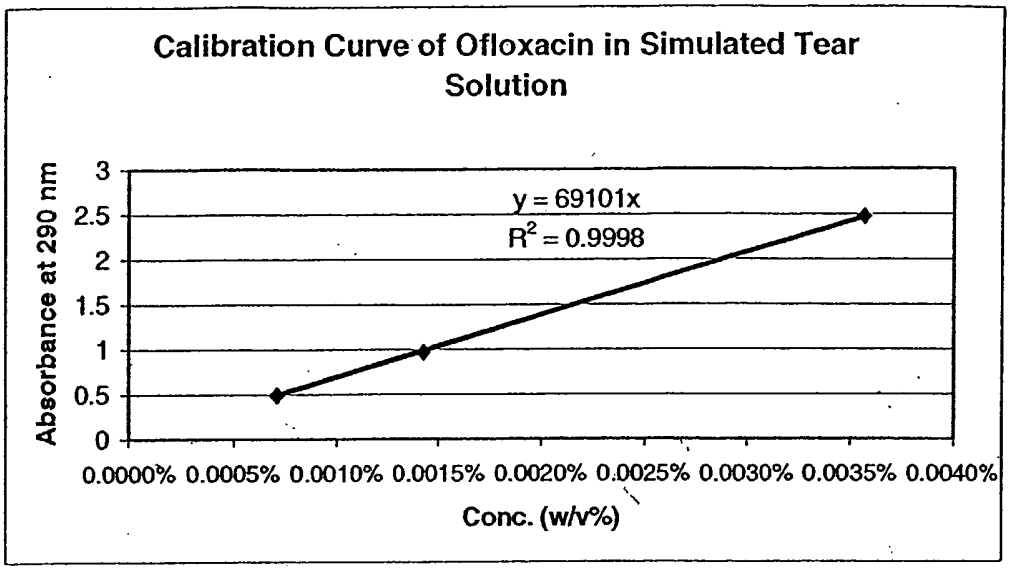


FIGURE 20

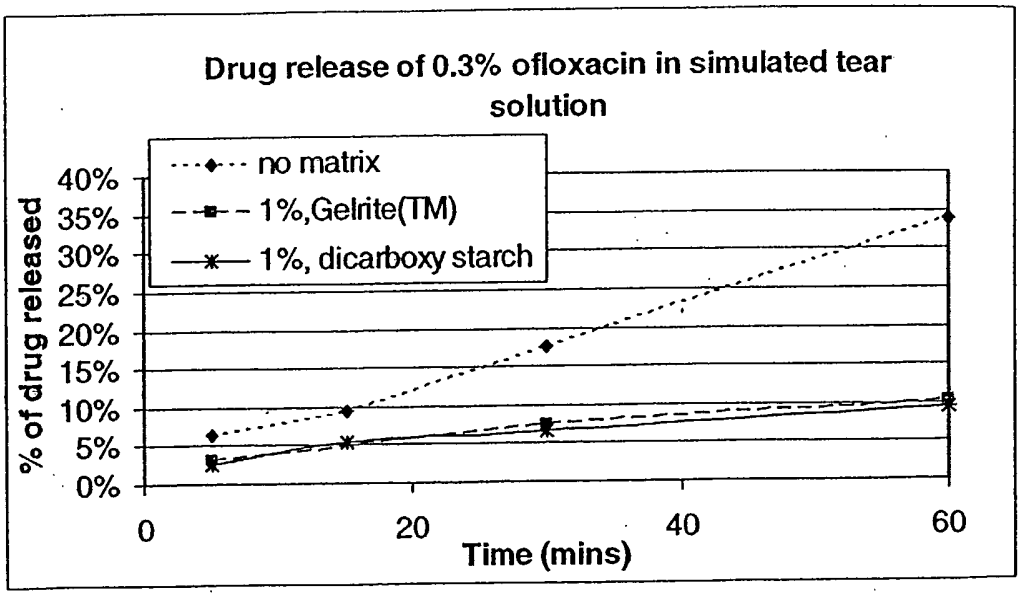


FIGURE 21

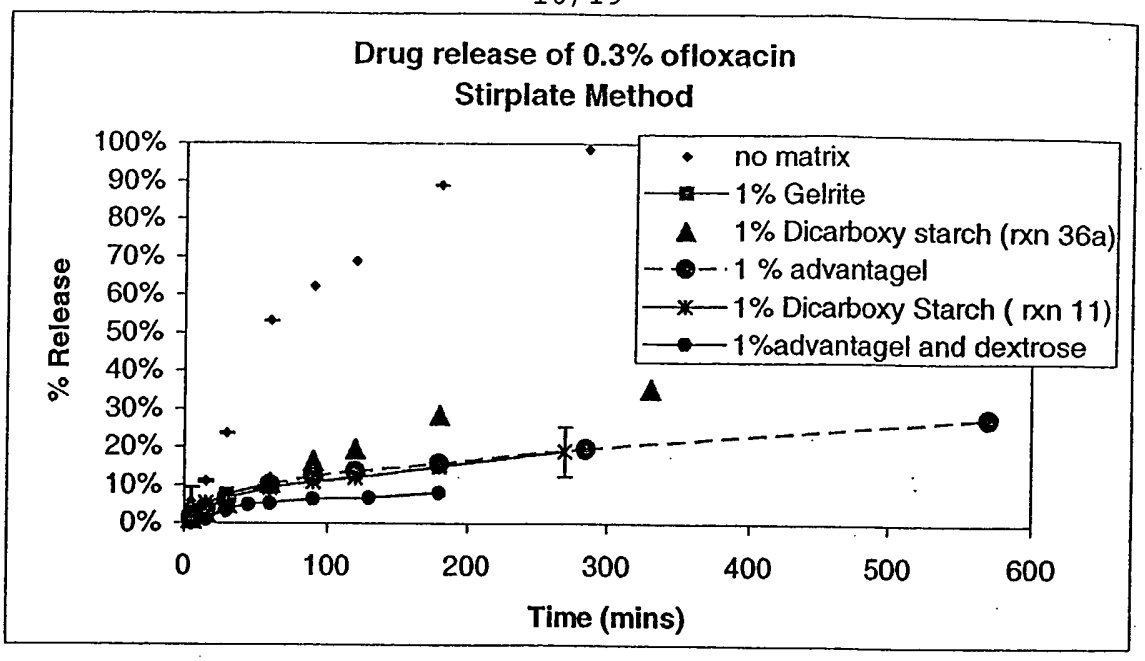


FIGURE 22

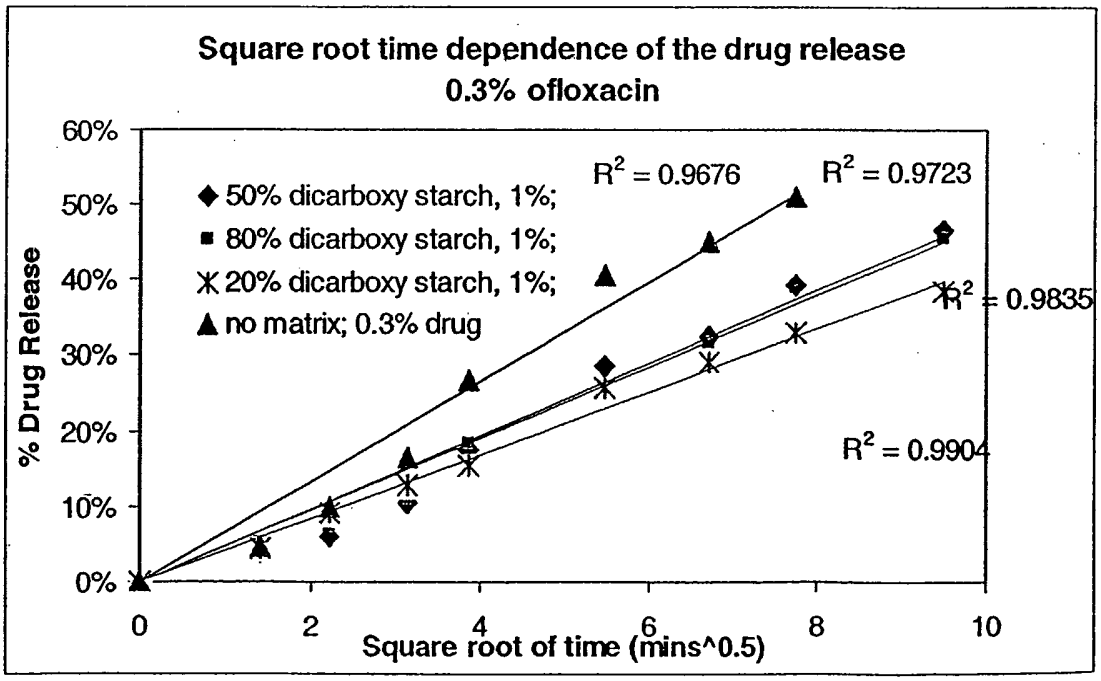


FIGURE 23

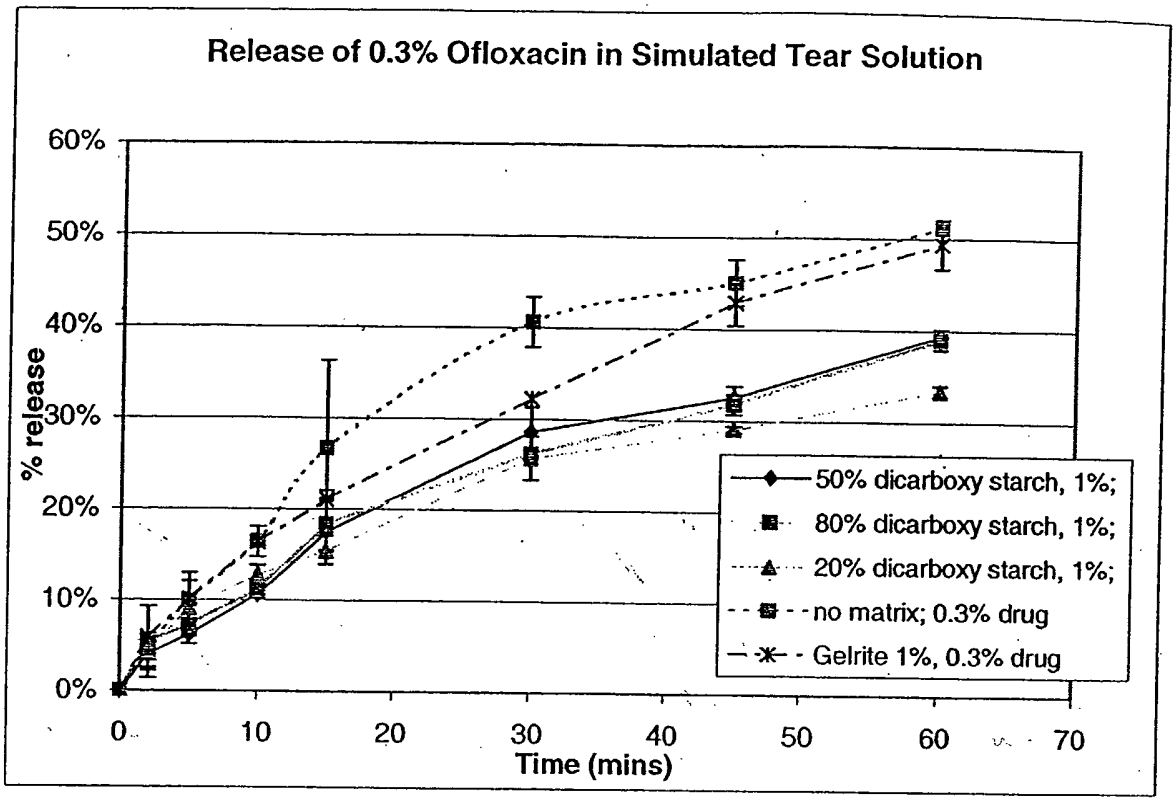


FIGURE 24

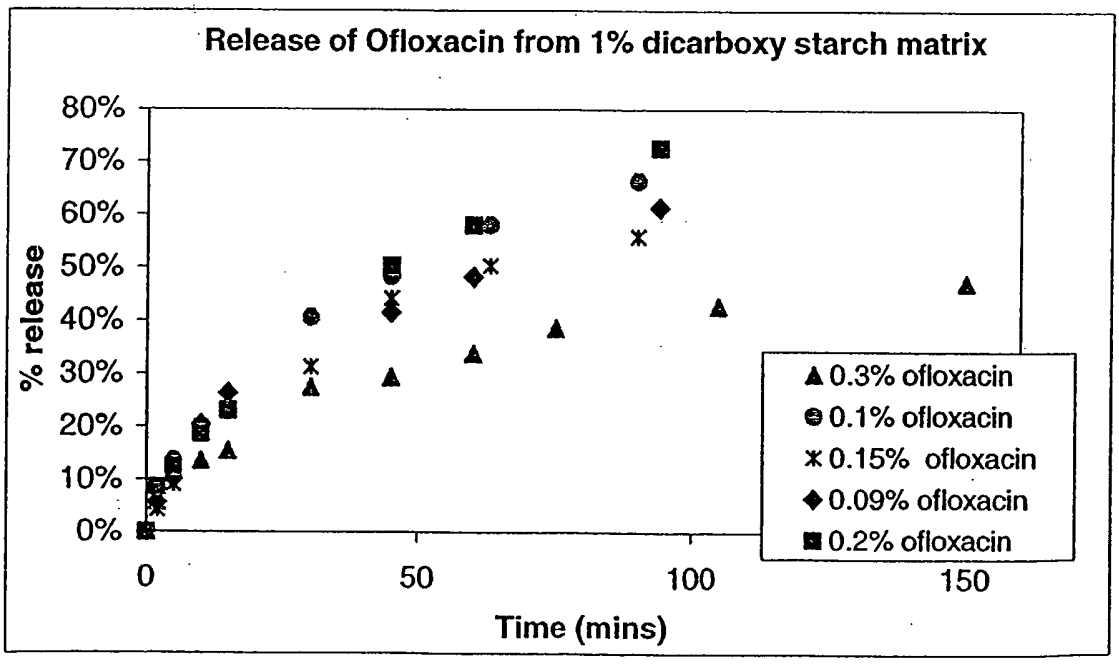


FIGURE 25

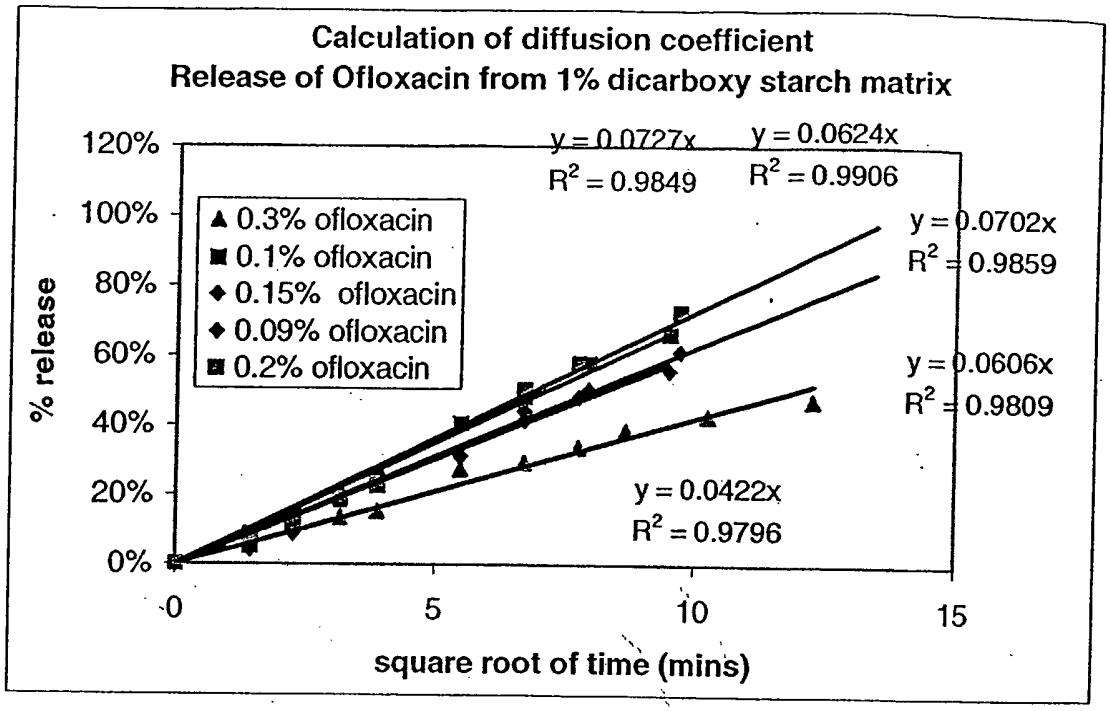


FIGURE 26

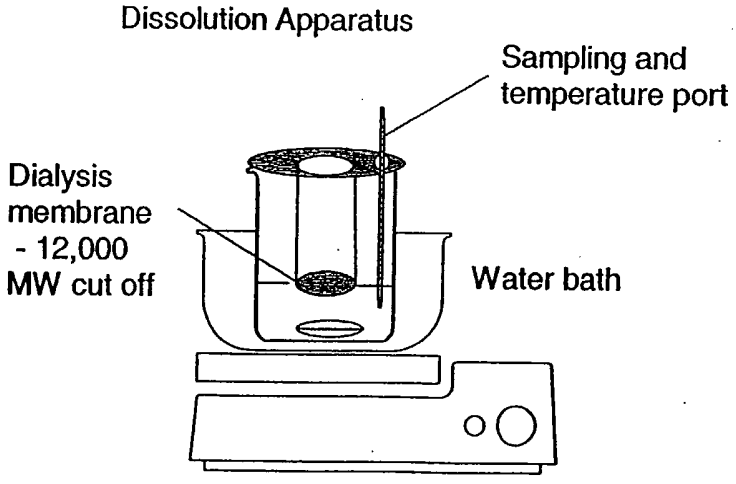


FIGURE 27

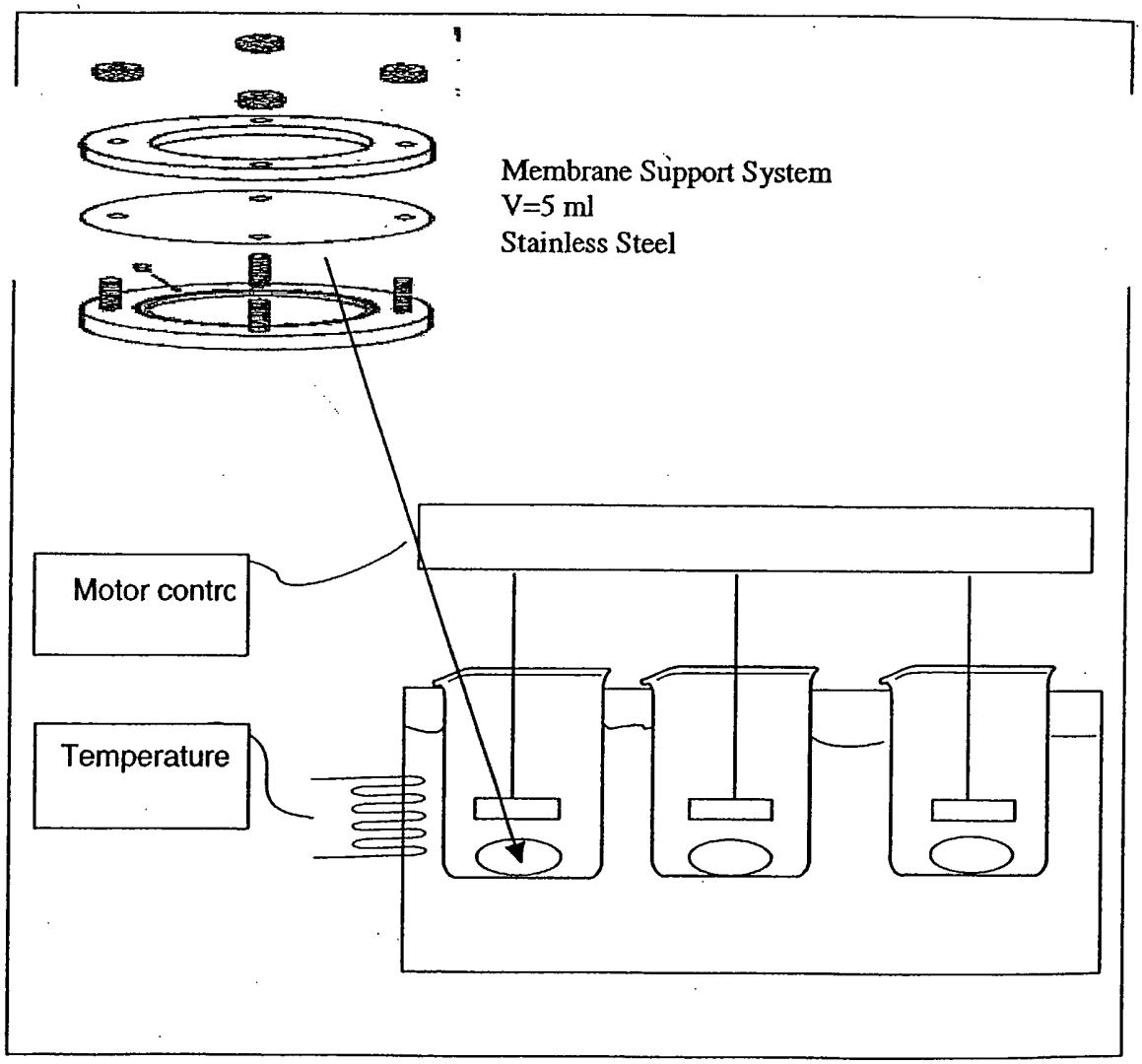

Membrane Support System
V=5 ml
Stainless Steel
Motor contrc
Temperature

FIGURE 28

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US05/16154

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/70
US CL : 514/25
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/25

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubMed, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Marsh et al. Isolation and Characterization of a Novel Acidic Polysaccahride Containing Tartrate and Glyoxylate Residues from the Mineralized Scales of a unicellular Coccolithophorid Alga Pleurochrysis cartae. Journal of Biological Chemistry. 1992, 267, 28 pp. 20507-20512. see figure 7 on p. 20512.	1-10
A	Lin et al. In Situ Gelling of Alginate/Pluronic Solutions for Opthalmic Delivery of Pilocarpine. Biomacromolecules. 2004. 5., pp. 2358-2365. See p. 2359 first column, first full paragraph second and third sentences.	1-10
A	Miyazaki et al. In Situ Gelling Xyloglucan Formulations for Sustained Release Ocular Delivery of Pilocarpine Hydrochloride. International Journal of Pharmaceutics. 2001., 229 pp. 29-36. See entire article and in particular p. 30 first full paragraph.	1-10
A	Carlfors et al. Rheological Evaluaion of Gelrite in Situ Gels for Ophthalmic Use. European Journal of Pharmaceutical Sciences. 1998., 6, pp. 113-119. See entire article and in particular p. 117 second column.	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search
09 July 2005 (09.07.2005)

Date of mailing of the international search report
12 OCT 2005

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