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Research Article

Effect of EDTA and Methionine on Preventing Loss of Viscosity of Cellulose-Based Topical Gel

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Abstract. Methylcellulose and hydroxypropylmethylcellulose (hypromellose) are used in topical formulations of a protein to form a viscous hydrogel. Five lots of hypromellose raw material were made into 3% gel; all showed viscosity loss after sterilization by autoclave. EDTA (edetate disodium) minimized the viscosity loss caused by autoclaving in the presence of up to 100 ppm H_2O_2. These results suggest that EDTA may prevent loss of viscosity of the hydrogel when peroxide is present. H_2O_2 at low levels (2–50 ppm) caused significant viscosity loss over time at either 40°C or 5°C in 3% methylcellulose or hypromellose gel. EDTA slowed the rate of viscosity loss during storage under stress by H_2O_2 but did not completely prevent the loss. Methionine was effective in completely preventing gel-viscosity loss during storage in the presence of up to 50 ppm H_2O_2. On the basis of these results, it is recommended that methionine be added to the protein topical formulation as a stabilizer against viscosity loss.

KEY WORDS: autoclave; EDTA; HPMC; hydrogel; hypromellose; methionine; methylcellulose; protein; viscosity.

INTRODUCTION

Growth factors such as fibroblast growth factors (acidic and basic), epidermal growth factor, vascular endothelial growth factor, platelet-derived growth factor (Regranex Gel®), etc. have been investigated as topically applied therapeutics for treatment of diabetic skin ulcer or wound healing. For this kind of application, the dosage form is often a hydrogel. These topical formulations may contain either methylcellulose USP/NF (Methocel® A4M) or hydroxypropylmethylcellulose USP/NF (hypromellose, Methocel® E4M) as the gelling agent. Methylcellulose and hypromellose share the same cellulose backbone but differ in the substitution of certain hydroxyl (HO–) groups by methoxyl (CH_3O–) in the case of methylcellulose and hydroxypropyl ($CH_3CH(OH)CH_2O$–) plus methoxyl groups in the case of hypromellose.

In pharmaceutical preparations, cellulose derivatives are widely used to impart viscosity to topical, ophthalmic, and vaginal formulations. The stability of the viscosity of these cellulose-based hydrogels at various pH levels and temperatures has been studied (1–3). In addition to acid hydrolysis and breakdown by high temperatures, oxidative degradation has also been suspected to cause loss of gel viscosity (4). Viscosity loss in hydroxyethylcellulose (HEC)-thickened latex paint has been attributed to chemical oxidants through measurement of the oxidation reduction potential (5). In addition, it has been reported that the thermal degradation temperatures and activation energies for cellulose ethers such as methylcellulose, ethylcellulose, sodium carboxymethylcellulose, HEC, and hypromellose are usually lower in air than in nitrogen (6).

When a hydrogel is sterilized, the gel is exposed to high temperatures, which may cause viscosity loss. Chu and Doyle (7) reported significant viscosity loss of the sodium carboxymethylcellulose gel used for compounding platelet-derived growth factor (in becaplermin [Regranex Gel®]) when the autoclave tank was flushed with oxygen, whereas viscosity was well maintained when the tank was flushed with nitrogen. Although sterility is not claimed for Regranex, it is nonetheless produced with autoclave sterilization to minimize bioburden. Autoclave sterilization of cellulose derivatives is not limited to topical products applied to open wounds. Numerous ophthalmic products contain cellulose derivatives to increase viscosity, and they too are autoclave-sterilized.

Recognizing the role that oxidation plays in the stability of cellulose, Dahl and coworkers (8) studied the effect of spiked hydrogen peroxide (H_2O_2) at 20 and 200 ppm on an HEC-based gel and observed a rapid reduction in gel viscosity. They also reported that butylated hydroxyanisole had a marginal protective effect, as an anti-oxidant, on gel viscosity.

Recently, hydroxypropylcellulose (HPC) from different lots was found to contain substantial amounts of oxidant, equivalent to 50–890 nmol hydroperoxide per gram of HPC, with significant lot-to-lot and manufacturer-to-manufacturer variations (9). In the case of HPC, peroxide can be extracted into the aqueous medium without an interfering gel phase,

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ABBREVIATIONS: EDTA (disodium salt), Ethylenediamine tetraacetate; HEC, Hydroxyethylcellulose; HPC, Hydroxypropylcellulose.

and the peroxide level can be determined. However, the exact level of peroxide in methylcellulose or hypromellose cannot be determined by the same method because once the cellulose derivatives are placed in water, they are hydrated and become a viscous liquid or gel that renders the assay inoperable. Because these cellulose derivatives are made in a similar manner, one may expect that the peroxide level in HPC may be extended to methylcellulose or hypromellose. Hydroperoxide at 890 nmol/g would result in 0.89 ppm of the peroxide in a gel when 3% of the cellulosic gelling agent is used in formulation.

There is further evidence of the presence of oxidant in a cellulose-based preparation and of its possible effect on protein stability. Nguyen (10) reported in the early 1990s that relaxin was oxidized much faster in methylcellulose gel than in aqueous solution. Therefore, the concern related to the presence of oxidant in cellulose derivatives is not just limited to viscosity loss, but also to the degradation of any oxidation-sensitive drug substance in the formulation.

In this work, we investigated accelerated oxidative damage to cellulose gels by spiking in various levels of H_2O_2 and monitoring viscosity loss. Levels of H_2O_2 from 2 to 20 or 100 ppm were used to induce an oxidative reaction. These levels are higher than those described in the aforementioned report on HPC (9)—for example, 0.96 ppm peroxide when made into a 3% gel. The evaluation of higher levels, up to 20–100 ppm of H_2O_2, can be justified because autoclaving can generate additional amounts of reactive oxidizing species and because other excipients such as polysorbate or polyethylene glycol may also introduce additional peroxides. The study described in this report examined the effect of EDTA on viscosity loss during autoclave sterilization of hypromellose gel in the presence of H_2O_2 and the effect of EDTA and methionine, alone or in combination, on viscosity loss during long-term storage (6 months) in the presence of H_2O_2.

MATERIALS AND METHODS

Materials

Methylcellulose USP/NF (Methocel® A4M) and hypromellose USP/NF (Methocel® E4M, 4 Lots TK, UF, UI, and TH) were obtained from Dow (Midland, MI). Hypromellose lot OS (the fifth lot) was prepared and tested by an oversea supplier. Sodium succinate 6-hydrate and succinic acid were purchased from JT Baker (Phillipsburg, NJ). H_2O_2 (30% solution) was purchased from VWR International (West Chester, PA) and EDTA and methionine from Sigma-Aldrich (St. Louis, MO).

Methods

Preparation of Gels

For safety, all autoclaved bottles were fitted with a cap having a filtered vent.

Gels from various lots of hypromellose. Two hydrogels were prepared from each of four lots of hypromellose powder. One gel of each lot was autoclaved and one was not. Sodium succinate (5 mM, pH 5.0) buffer was weighed into 500-mL Kimax bottles and heated to 70°C. Hypromellose powder was added to make a 4.7% (*w*/*w*) gel. The exact amount of powder added was determined by weight to ensure that any variation was not due to differences in powder transfer. The powder was dispersed into the buffer to form slurry by swirling the bottle. One bottle of each lot of gel to be tested was then autoclaved for 45 min at 121°C and 15 psi. After the autoclave cycle, the bottles were shaken to disperse the precipitated hypromellose and then weighed. Buffer was added to all bottles (autoclaved and not autoclaved) to make 3% gels (based on powder weight). The bottles were then placed on a bottle roller at 2–8°C until the appearance of the gel was homogeneous.

Addition of H_2O_2 and EDTA to gels before autoclave heat treatment. One stock gel for hypromellose and one for methylcellulose were prepared. Sodium succinate (5 mM, pH 5.0) buffer was heated to 70°C. Gel powder was then added to make a 4.7% gel. The suspension was mixed with a Heidolph RZR 2021 propeller mixer until it formed a uniform hydrogel. The gel was then allowed to sit at room temperature until all the bubbles had risen out of it. Ten grams of gel was then weighed into 100-mL Kimax bottles. Aliquots of 1,000 ppm EDTA stock solution, 3,000 ppm peroxide stock solution, or both were added to each gel to achieve the desired final concentrations. The gels were autoclaved for 20 min at 121°C and 15 psi. The bottles were shaken after the autoclave cycle to disperse the precipitated methylcellulose or hypromellose and then placed on bottle rollers at 2–8°C. To study the effect of autoclaving, these samples were tested without further storage.

Preparation of Gels for Storage Studies. One stock gel for hypromellose and one for methylcellulose were prepared for each study. Sodium succinate (5 mM, pH 5.0) buffer was heated to 70°C. Gel powder was then added to make a 4.7% gel. The suspension was mixed with a Heidolph RZR 2021 propeller mixer until it formed a uniform hydrogel. The gels were then transferred to Kimax bottles and autoclaved for 45 min at 121°C and 15 psi. The bottles were shaken after the autoclave cycle to disperse the precipitated methylcellulose or hypromellose and then placed on bottle rollers at 2–8°C.

Different formulations of each gel type were made by transferring and weighing approximately 13 g of 4.7% gel into 50-mL polypropylene tubes. Buffer alone, buffer with 640 ppm EDTA, buffer with 5 mg/mL methionine (not used prior to autoclaving), or buffer with EDTA and methionine was added for a final concentration of 3% gel, with 400 ppm EDTA and 1.8 mg/mL methionine in the appropriate gels. To these gels, 3,000 ppm stock H_2O_2 was added to a 0, 2, 5, 20, or 50 ppm concentration. The gels were then mixed at 2–8°C on a bottle roller. After mixing, 1 g of each gel was dispensed into a 5 cc West glass vial with a serum stopper. The vials were stored at 5°C and 40°C for designated period of time.

Viscosity measurements. Viscosity was determined using a Paar Physica UDS 200 cone and plate rheometer at a constant shear rate of 120 s^{-1} (about 20 rpm) using a 25-mm cone, temperature controlled to 25°C. A positive-displacement pipette was used to put approximately 200 μL of gel on the

plate; excess was removed once the cone was lowered into measuring position. The average value of all readings at half minute intervals over a span of 10 min was taken as the viscosity measurement. Typically, three independent measurements were taken for each sample. Each reported viscosity value was the average of three measurements for each sample. In general, without added H_2O_2, the viscosity of methylcellulose and hypromellose gels was about 2,800 cP under the measurement conditions (shear rate, 120 s^{-1}).

RESULTS AND DISCUSSION

Viscosity Loss Due to the Autoclave Sterilization Process

Viscosity loss due to the autoclave sterilization process has previously been observed with sodium carboxymethylcellulose (7). We chose to study uncharged cellulose derivatives such as methylcellulose and hypromellose because of concerns about potential interactions between charged cellulose derivatives and protein. To evaluate the susceptibility of hypromellose to autoclave sterilization, four lots of domestic hypromellose raw material (lots TK, UF, UI, and UH) and one lot from oversea were made into 3% gel, and the gel viscosity before and after autoclave sterilization was assessed. All five gels showed viscosity loss to some extent (Fig. 1), and the gel made from oversea hypromellose lost the most viscosity after autoclave sterilization.

Effect of EDTA on Autoclave-Induced Viscosity Loss in the Presence of Peroxide

An attempt to use butylated hydroxyanisole as an anti-oxidant in HEC gel to prevent viscosity loss was not successful (8). There are many anti-oxidants that can be used to inhibit the oxidation of pharmaceuticals. However, majority of these anti-oxidants are prone to oxidation during autoclaving at high temperature and high pressure and thus are unsuitable for any preparation need be autoclaved. EDTA and certain analogues, because of their simple chemical structure and stability at elevated temperature, are the exceptions. For this reason, viscosity stabilization by EDTA was evaluated. In the absence of EDTA, the loss of viscosity increased with increasing amounts of H_2O_2 for both methylcellulose and hypromellose gels (Fig. 2). As shown in the same figure, when EDTA was added at 400 ppm to gels containing H_2O_2, the viscosity loss was minimized. The benefit of EDTA was more prominent at higher concentrations of H_2O_2 than at lower concentrations.

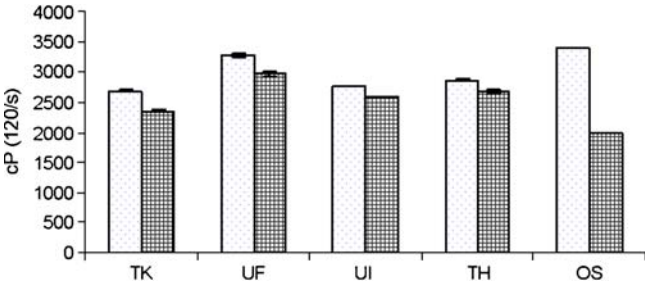

Fig. 1. Viscosity of 3% hypromellose gels before (*dotted column*) and after (*grid column*) autoclave sterilization. Each gel was made from a different lot of hypromellose powder (*TK*, *UF*, *UI*, *TH*, or *OS*)

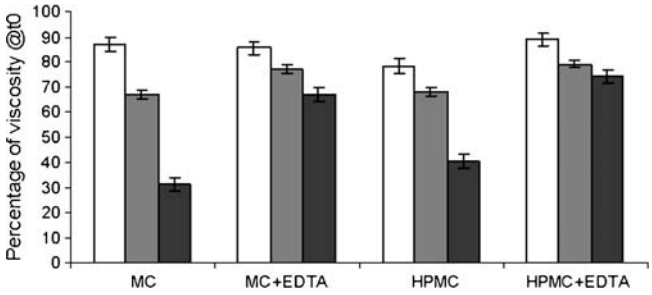

Fig. 2. Percentage of viscosity remaining in the absence and presence of EDTA after autoclaving. H_2O_2 at 1 ppm (*white bars*), 10 ppm (*gray bars*), and 100 ppm (*black bars*) caused the gel-viscosity loss. *MC* methylcellulose, *HPMC* hypromellose

Effect of Hydrogen Peroxide Concentration and Temperature on Gel-Viscosity Loss during Storage

In this study, the effect of different levels of H_2O_2 on gel viscosity during storage at 5°C and 40°C was studied. Figure 3a and b shows that H_2O_2, at concentrations at or above 5 ppm, caused a rapid, substantial decrease in the viscosity of hypromellose gel. The higher the concentration of H_2O_2, the greater was the loss of gel viscosity. At 5°C (Fig. 3a, where the scale is shown in weeks), the percentage of remaining viscosity at week 8 was 70%, 45%, 40%, and 40% for the gels containing 2, 5, 20, and 50 ppm H_2O_2, respectively. The decrease at 40°C was much faster than that at 5°C. At 40°C (Fig. 3b, where the scale is shown in days),

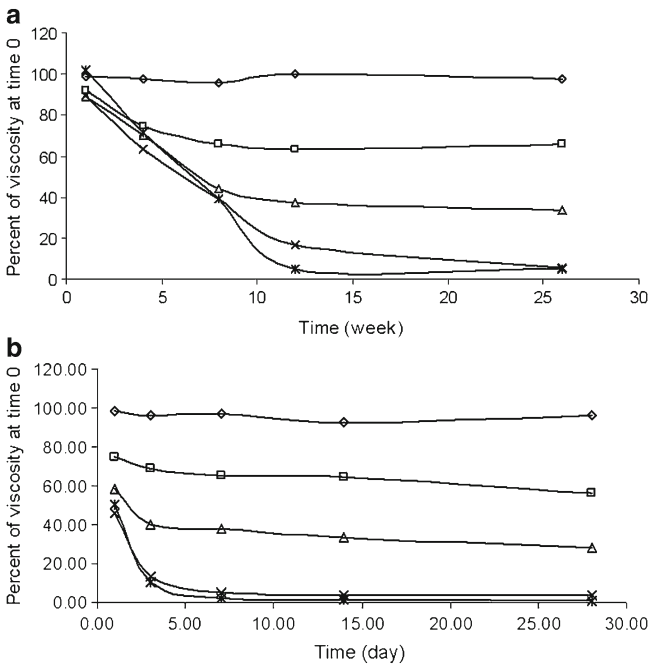

Fig. 3. Percentage of viscosity remaining relative to time zero in 3% hypromellose gel in the presence of various concentrations of H_2O_2 at 5°C (**a**) and 40°C (**b**). *Diamonds* 0 ppm H_2O_2, *squares* 2 ppm H_2O_2, *triangles* 5 ppm H_2O_2, *multiplication signs* 20 ppm H_2O_2, *asterisks* 50 ppm H_2O_2

the percentage of remaining viscosity on day 3 compared to that at time zero was 70%, 40%, 15%, and 10% for the gels containing 2, 5, 20, and 50 ppm H_2O_2, respectively. The decrease in viscosity at 40°C reached a plateau after 3 days, while the viscosity of the gels at 5°C with 20 and 50 ppm H_2O_2 continued to decrease. The viscosity loss of the gels with 2 and 5 ppm H_2O_2 at 5°C reached a plateau after 12 weeks. The explanation for this observation is that, concurrent with the loss of viscosity, the H_2O_2 concentration in the gel also decreased. Although peroxide was not measured, one may expect that when the gel was spiked with a large amount of H_2O_2, it took more time for the concentration of H_2O_2 to decrease to a very low level at 5°C than it did at 40°C. After 8 weeks, there was still a fair amount of H_2O_2 residue in the gel at 5°C, and the viscosity of the gel continued to decrease.

Figure 4a and b shows the percentage viscosity remaining in the methylcellulose gels in the presence of different levels of H_2O_2 at 5°C and 40°C. Phenomena very similar to the hypromellose gels were observed. The slight increase in viscosity observed in the sample containing 0 ppm H_2O_2 is experimental error; the percent error of the viscosity measurement is about ±10%.

Both hypromellose and methylcellulose contain a polymeric backbone of cellulose, a natural carbohydrate that contains a basic repeating structure of anhydroglucose units. Gel-viscosity loss is due mainly to the cleavage of the glycosidic linkage of the cellulose ether by hydroxyl radicals (11). One of the mechanisms proposed is that the C–H bonds

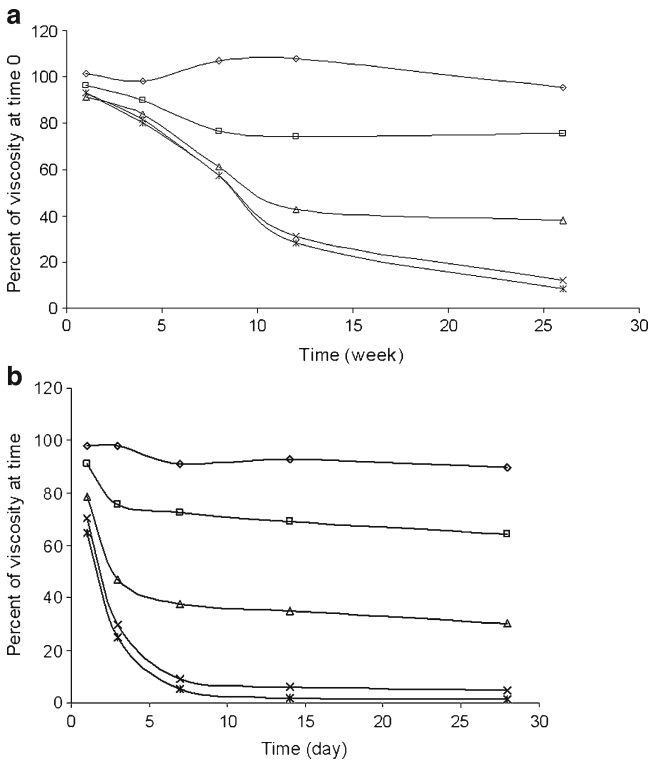

Fig. 4. Percentage of the viscosity remaining relative to time zero in 3% methylcellulose gel in the presence of various concentrations of H_2O_2 at 5°C (**a**) and 40°C (**b**). *Diamonds* 0 ppm H_2O_2, *squares* 2 ppm H_2O_2, *triangles* 5 ppm H_2O_2, *multiplication signs* 20 ppm H_2O_2, *asterisks* 50 ppm H_2O_2

are first oxidized, in a stepwise process, to form hydroperoxide (–C–OOH). Decomposition of the hydroperoxide formed at the acetal C–H can cleave the main polymer chain and cause a significant decrease in gel viscosity (8,12). More recently, using 1, 4-anhydrocellobitol as the model compound, it was found that the hydroxyl radicals can simply cleave any randomly encountered glycosidic linkages in cellulose and that the main degradation mechanism is primarily substitution reactions of hydroxyl radicals at the anomeric carbon (13).

Effect of EDTA and Methionine on Gel-Viscosity Loss during Storage

Given that EDTA exhibited stabilization of viscosity loss caused by the autoclave sterilization process (Fig. 2), it was hypothesized that EDTA may also stabilize viscosity loss during storage in the presence of added peroxide. In addition to EDTA, a useful anti-oxidant, methionine, was considered since it can be added to a hydrogel after autoclaving. Therefore, the effects of EDTA and methionine on the stability of gel viscosity during storage were investigated.

The experiment was carried out at 40°C for 4 weeks (Fig. 5) and at 5°C (Fig. 6) for 6 months. At 40°C and without added EDTA or methionine, gel viscosity decreased significantly in the presence of 2, 5, 20, or 50 ppm H_2O_2, as discussed previously and shown in Fig. 3. Figure 5a shows that EDTA slowed the rate of viscosity loss (Fig. 3b). However, EDTA alone could not completely prevent viscosity loss during storage at 40°C. When methionine was added to the gel at a concentration of 1.8 mg/mL, it was sufficient to prevent viscosity loss during storage at 40°C even in the presence of 50 ppm H_2O_2 (Fig. 5b). Figure 5c shows that when both EDTA and methionine were present in the gel, gel viscosity was also maintained. Because methionine alone provided excellent protection, the results shown in Fig. 5c do not allow the effect of adding EDTA in this case to be discerned.

Figure 6a–c shows the stability of gel viscosity during storage at 5°C in formulations containing EDTA, methionine, or both, respectively, in the presence of different levels of H_2O_2. In results similar to those obtained at 40°C, methionine at 5°C effectively prevented the gel-viscosity loss caused by H_2O_2. However, the data in Fig. 6a indicate that EDTA alone prevented viscosity loss at 5°C. Unlike the observations at 40°C (Fig. 5a), the protection at 5°C was significant when a comparison is made between the absence of EDTA (Fig. 3a) and the presence of EDTA (Fig. 6a). Since EDTA appears to have a protective effect, it is possible that a free-radical reaction is occurring. Because EDTA sequesters and inactivates the trace metals that catalyze free-radical reactions, EDTA is considered a stabilizer against such reactions. It is possible that at 5°C the generation of free radicals is slow, allowing EDTA the chance to exert its sequestering effect on the catalytic metals and thus minimize the quantity of free radicals generated. Consequently, loss of viscosity for gels stored at 5°C can be minimized by the addition of EDTA. In contrast, at 40°C, free-radical generation is much faster and sequestration of metal by EDTA cannot reduce the formation of free radicals, and thus EDTA exhibits a minimal effect.

The use of H_2O_2 to stress a gel made of cellulose derivatives is well founded because H_2O_2 has been found in

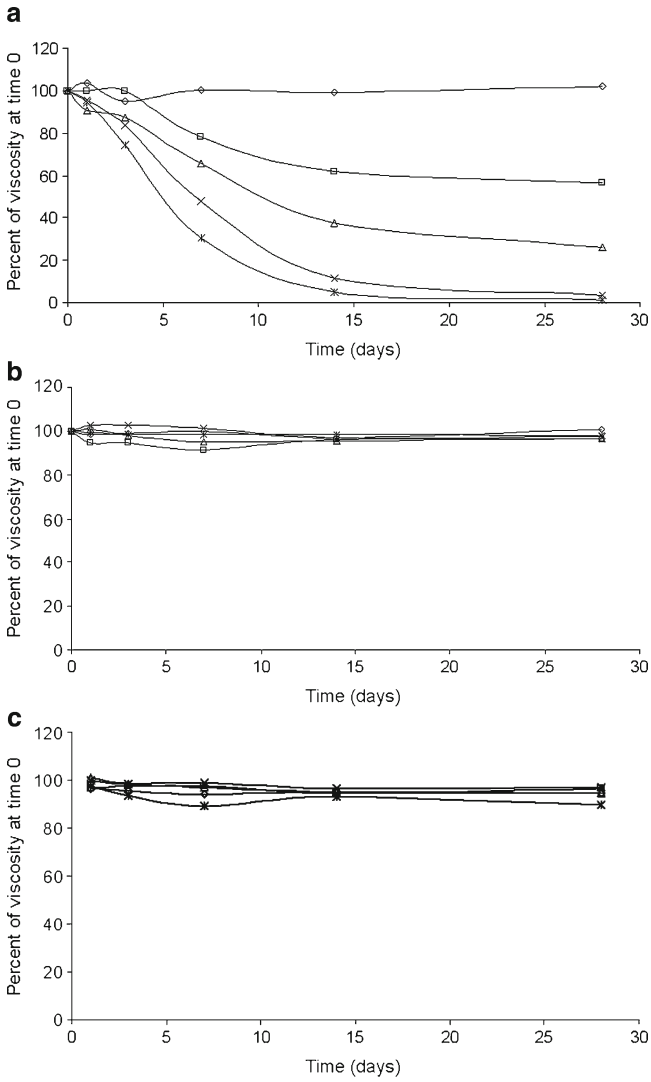

Fig. 5. Percentage of viscosity remaining relative to time zero in 3% hypromellose gel in the presence of 400 ppm EDTA (**a**), 1.8 mg/mL methionine (**b**), and both 400 ppm EDTA and 1.8 mg/mL methionine (**c**) and various concentrations of H_2O_2 at 40°C for 4 weeks. *Diamonds* 0 ppm H_2O_2, *squares* 2 ppm H_2O_2, *triangles* 5 ppm H_2O_2, *multiplication signs* 20 ppm H_2O_2, *asterisks* 50 ppm H_2O_2

various sources of cellulose raw material (9). The appropriate level of peroxide to use in a stressed study is debatable. In this study, a wide range of peroxide concentrations, from 2 to 50 ppm, was used. According to a previous report (9), 2 ppm may be present in cellulose raw material when it is made into a 3% gel. Since autoclaving may generate additional free radicals and peroxide, 50 ppm may be the likely level for a gel at the initiation of long-term storage. Therefore, 2–50 ppm could be an appropriate and realistic range for evaluation. The results and recommendations made from the study should lead to a robust and stable formulation.

Figures 5 and 6 show methionine to be an effective stabilizer against viscosity loss of a hypromellose gel stressed by H_2O_2. EDTA exhibits a marginal stabilization effect under storage conditions. Based on these results, methionine is a recommended component for a cellulose-containing formulation when stored after autoclave sterilization.

CONCLUSIONS

Hypromellose gels made from five lots of raw material were found to show viscosity loss after autoclave sterilization. Detectable viscosity loss occurred in the presence of various levels of H_2O_2 (e.g., 1–100 ppm) in hypromellose and methylcellulose gels. EDTA minimized the viscosity loss in the presence of H_2O_2 during autoclaving.

During gel storage at 40°C, EDTA slowed the rate of viscosity loss but did not completely prevent it. At 5°C, EDTA showed better protection. Methionine was effective in completely circumventing the loss of gel viscosity during storage at 5°C or 40°C when the gel was stressed by 2–50 ppm H_2O_2. Based on this finding, methionine may be considered for use as a gel-viscosity stabilizer in cellulose-containing formulations.

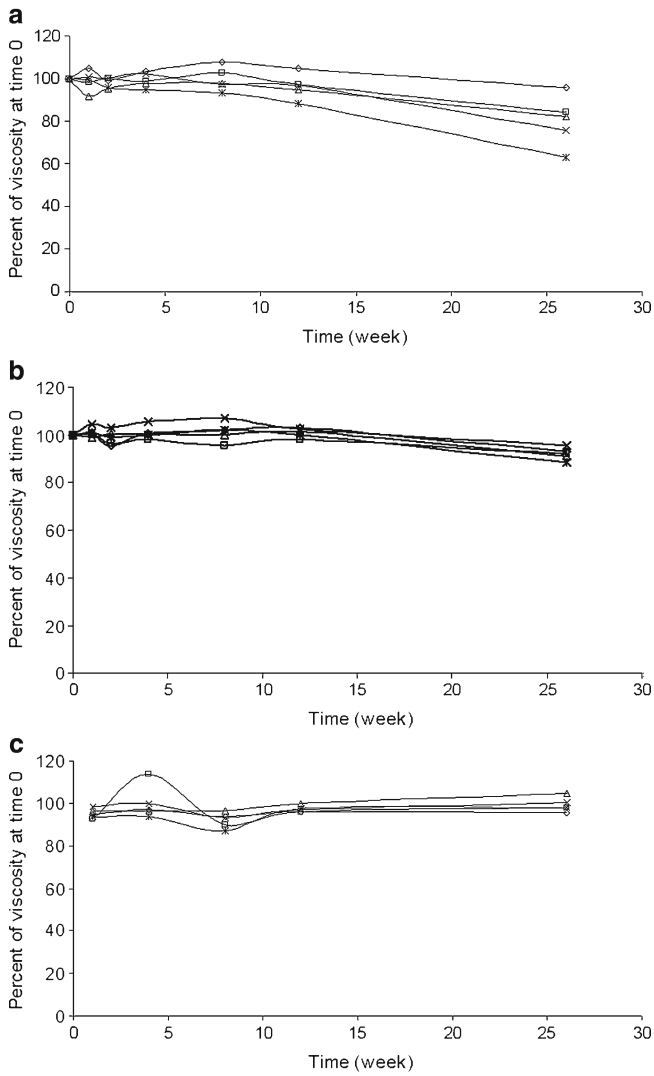

Fig. 6. Percentage of viscosity remaining relative to time zero in 3% hypromellose gel in the presence of (**a**) 400 ppm EDTA, (**b**) 1.8 mg/mL methionine, and (**c**) both 400 ppm EDTA and 1.8 mg/mL methionine and various concentrations of H_2O_2 at 5°C for 6 months. *Diamonds* 0 ppm H_2O_2, *squares* 2 ppm H_2O_2, *triangles* 5 ppm H_2O_2, *multiplication signs* 20 ppm H_2O_2, *asterisks* 50 ppm H_2O_2

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