Original Article

TIPS Trends in Pharmaceutical Sciences 2022: 8(3): 175-182.

# The Effects of Moist Heat Sterilization Process on Rheological Properties of Hydrophilic gels containing drug model

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## Abstract

Wound healing is a complex cascade of cellular and biochemical actions. Numerous methods, such as application of topical antimicrobial therapies and wound dressings have been reported to accelerate healing process. One important factor in all mentioned methods is the sterility of the delivery or dressing system. In this study, the effect of steam sterilization on the viscosity of polymeric gels of hydroxyl propyl methyl cellulose, sodium carboxy methyl cellulose, carbomer, and sodium alginate was evaluated. The results showed that carbomer (0.5%) and hydroxyl propyl methyl cellulose (3%) had proper rheological behavior and could be used as gel bases in the rest of the study. In addition, the effect of solubility enhancers on the properties of hydrophilic gels was examined. Tween 80 (0.1 and 1%) and propylene glycol (1 and 10%) were examined as solubility enhancers. The proper gel was prepared from carbomer and hydroxyl propyl methyl cellulose containing 0.1 % tween 80 or 1% propylene glycol. In the next step, Atorvastatin, as a promising wound healing drug model, was added to selected bases to evaluate the rheological properties of the gel. Content uniformity of the drug in the gels was examined either. The results revealed that content uniformity maintained constant in carbomer gel after sterilization. As a result, carbomer could be a proper base to deliver drugs to affected areas after sterilization.

*Keywords*: Carbomer, Hydroxyl Propyl Methyl Cellulose, Sodium Alginate, Sodium Carboxy Methyl Cellulose, Sterile, Viscosity.

Please cite this article as: Negar Negahban, Shohreh Alipour, Fatemeh Ahmadi, Elahehnaz Parhizkar. The Effects of Moist Heat Sterilization Process on Rheological Properties of Hydrophilic gels containing drug model. Trends in Pharmaceutical Sciences. 2022;8(3):175-182. doi: 10.30476/TIPS.2022.94633.1138

## 1. Introduction

Hydrophilic gels are three-dimensional cross-linked networks of aqueous liquid that is entrapped by colloidal solid particles. Many types of pharmaceutical materials can be used in the preparation of hydrophilic gels. One of the most applicable excipients are polymers. Polymeric gels are of the most attractive topical delivery systems for topical or systemic effects. They have been accepted to deliver the active pharmaceutical ingredients directly to the topical layer of the skin, from where it gets absorbed into the blood circulation (1-3).

Skin is the most exposed organ to damage and injury. By damaging the structures of skin, a cascade of phases, known as wound healing or wound repair starts in the body (4, 5).

Hydrophilic gels are expected to maintain a moist environment around the wound and absorb the wound exudates. As hydrophilic gels swell upon hydration, they provide the necessary trapped moisture for wound healing (6, 7).

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Received: 12/03/2022; Accepted: 01/09/2022

Sterility is one of the most important characteristics of hydrophilic gels and other wound dressings that are intended for application on wounded skin (8).

The most common difficulty in wound healing is prevention of an infection, especially in chronic wounds. Bacteria are a common part of the intact skin microbiota and even wounds. But a critical threshold of existing bacteria and the formation of a biofilm may impede wound healing (9, 10).

As a result, effective wound dressing would decrease the bioburden in the wounded area and results in improved therapeutic outcomes (11, 12).

The main aim of this work is to determine the rheological behavior of hydrophilic gels as carriers for drug delivery to wounds that can tolerate steam sterilization.

Heat, presence of inorganic ions, solubility enhancers (especially when active pharmaceutical ingredients have low solubility), concentration of the polymer, pH, and many other factors may change physical properties of hydrophilic gels, especially their rheological behavior and viscosity properties. In this study, the effect of autoclaving at 121 °C for 20 min (moist heat sterilization process) was evaluated. Hydrophilic gels were formulated from carbomer 934, low viscosity Sodium Carboxy Methyl Cellulose (Na CMC), Hydroxyl Propyl Methyl Cellulose (HPMC), and Sodium Alginate (Na Alg). In the next step, the effect of presence of two different types of solubility enhancers that are usually added to topical formulation was evaluated. Tween 80 and propylene glycol (PG) were selected as common enhancers (13) and the effect of their concentrations on the rheological behavior of the hydrophilic gels before and after sterilization was evaluated. At the end, Atorvastatin (ATR) was added to the selected base as a drug model that showed wound healing properties (14, 15) and the rheology of drug- containing gels was examined either.

## 2. Materials and methods

### *2.1. Materials*

HPMC 15 mPas, low viscosity Na CMC, Na Alg and carbomer 934 were obtained from Merck, Germany. ATR was purchased from Sobhan Co., Iran. All other chemicals were analytical grades.

### *2.2. Preparation of Hydrophilic gels*

Carbomer was dispersed in purified water by mixing on a magnetic stirrer, and after proper wetting, the dispersion was neutralized with a few drops of sodium hydroxide solution to pH 6.5.

HPMC and Na CMC were dispersed in purified water at temperature about 80 °C, and the mixture was cooled down to 4 °C and stirred until it reached homogenous hydrophilic gel.

Na Alg gel was prepared by dispersion of the polymer in purified water at room temperature and after complete dispersion, CaCl2 (1% W/V) was added.

The concentration of prepared hydrophilic gels is mentioned in Table 1.

### *2.3. Rheological Measurement*

The dynamic viscosity of prepared hydrophilic gels was measured 24 h after preparation using Viscometer R/S plus, Brookfield, USA, with a 25 mm cone at room temperature and shear rate of 100 1/s. Each sample was measured three times. All samples were allowed to equilibrate to room temperature before analysis.

### *2.4. Sterilization of hydrophilic gels*

Prepared hydrophilic gel samples were sterilized by autoclaving in glass vials at 121 °C

Table 1. Type and concentration of prepared hydrophilic gels.

| Polymer type | Prepared concentration (% w/v) | | |
|---|---|---|---|
| Carbomer | 0.25 | 0.5 | 1 |
| Na Alg | 2 | 3 | 4 |
| HPMC | 1 | 2 | 3 |
| Na CMC | 2 | 3 | 4 |

for 20 min. After sterilization, all samples were placed at room temperature and rheological parameters of sterilized samples were measured as it was mentioned in section 2.3.

### *2.5. Preparation and rheological measurement of Hydrophilic gels containing solubility enhancers*

Tween 80 and PG were selected as solubility enhancers because they resist autoclaving and are used in sterile products (parenteral and non-parenteral) (13). They were added to selected hydrophilic gels at the concentrations of 0.1 and 1% of Tween 80 and 1 and 10% (W/W) of PG. Rheology of all prepared samples was measured by the method mentioned in section 2.3 before and after autoclaving and the data was collected.

### *2.6. pH of selected base*

pH of selected bases was evaluated by Adwa, AD1000 pH meter, Hungary. Each test was performed in triplicate.

### *2.7. Content uniformity of sample drug before and after sterilization*

ATR (1% w/v) as a promising wound healing agent was added to selected bases before and after sterilization and content uniformity of the drug was assayed based on ATR analysis method.

#### *2.7.1. ATR analysis*

UV-Vis spectrophotometry method was used at maximum absorbance wavelength (247 nm) in ethanol: water 25: 75 for ATR quantification. Different ATR concentrations (10, 8, 6, 4 and 2 µg/ml) were prepared using the serial dilution technique. All concentrations were prepared on three different days. Each concentration was tested in triplicate. Calibration curve was validated by linearity, intraday and inter-day precision (reported as RSD%), accuracy, limit of detection (LOD) and limit of quantitation (LOQ).

#### *2.7.2. Content uniformity assay*

A defined amount of gel containing ATR was diluted in ethanol: water 25: 75 and the amount of ATR was examined by the analysis method (section 2.7.1).

### *2.8. Rheological measurement of hydrophilic gels containing ATR*

Rheology of selected gel was measured by the method mentioned in section 2.3 before and after autoclaving and the data was collected.

### *2.9. Statistical Study*

All data were analyzed by one-way ANOVA test with post hoc Tukey's. $P<0.05$ has been considered as significant statistically

## 3. . Results and discussion

### *3.1. Rheological measurements of hydrophilic gels before and after sterilization*

The rheological data were fitted to power law model (equation 1) in which, K is system consistency and power law exponents (n) as shear thinning factor (Equation 1.).

$$\eta = K.\gamma^{n-1} \quad \text{(Eq. 1)}$$

If $0<n<1$, the system shows pseudo plasticity and shear thinning behavior. As n approaches unity, shear thinning behavior decreases (16, 17).

In addition, apparent viscosity of each sample was determined by plotting viscosity against shear rate. All data are presented in Table 2 and Figure 1.

As it is clear in Figure 1, low viscosity Na CMC samples did not have proper viscosity and the viscometer could not measure the parameters by the mentioned method. This data is in accordance with previous studies (18, 19). As a result, this polymer was excluded from the rest of the study.

In all other samples, apparent viscosity and K were decreased after sterilization process however; as overall, it was not significant in carbomer (except 0.25% that the viscosity decreased to half of its amount) and HPMC samples ($p>0.05$). As a comparison, n value was the lowest in carbomer samples that showed more shear thinning behavior of carbomer gel. Carbomer 0.25% had very low viscosity and carbomer 1% showed the highest viscosity. As a result, carbomer 0.5% was selected as proper hydrophilic gel for the rest of study.

In Na Alg gel samples, viscosity decreased significantly after autoclaving in all concentrations. However, at the concentration 2%, the de-

Table 2. Apparent viscosity, n and K of prepared hydrophilic gels.

| Polymer type | Sterility property | Concentration (%) | Apparent viscosity (Pa.s) | n | K (Pa.s) |
|---|---|---|---|---|---|
| Carbomer | Non sterile | 0.25 | 6.31±0.51 | 0.17±0.01 | 13.09±0.63 |
| | | 0.5 | 10.35±0.32 | 0.13±0.03 | 21.99±2.48 |
| | | 1 | 67.94±0.10 | 0.16±0.00 | 127.03±0.00 |
| | Sterile | 0.25 | 3.32±0.34 | 0.17±0.00 | 5.01±1.63 |
| | | 0.5 | 8.84±0.21 | 0.23±0.00 | 18.84±0.00 |
| | | 1 | 59.11±0.70 | 0.17±0.00 | 124.09±0.00 |
| Na Alg | Non sterile | 2 | 11.82±0.12 | 0.59±0.02 | 28.11±1.96 |
| | | 3 | 14.72±0.39 | 0.57±0.00 | 33.1±0.18 |
| | | 4 | 17.21±0.42 | 0.56±0.01 | 36.81±2.70 |
| | Sterile | 2 | 0.93±0.05 | - | - |
| | | 3 | 3.98±0.23 | 0.85±0.03 | 5.62±0.69 |
| | | 4 | 6.64±0.23 | 0.70±0.03 | 11.6±1.17 |
| HPMC | Non sterile | 1 | - | - | - |
| | | 2 | 10.89±0.23 | 0.71±0.03 | 18.04±2.24 |
| | | 3 | 15.53±0.18 | 0.57±0.06 | 31.84±2.75 |
| | Sterile | 1 | - | - | - |
| | | 2 | 7.02±0.20 | 0.78±0.00 | 7.01±0.00 |
| | | 3 | 11.63±0.42 | 0.62±0.00 | 21.17±1.14 |

crease in viscosity was so great that the viscometer could not measure it by the mentioned method. Based on the low viscosity of Na Alg samples at concentrations 2 and 3 %, these samples were not used in the rest of the study. Reduction in viscosity after sterilization process was attributed to the reduction in the degree of polymerization of the alginate molecules after heat treatments (20).

In HPMC samples, viscosity could not be measured at the concentration of 1% before and after autoclaving. In addition, data of 2% sample was not proper and as a result, HPMC 3% was

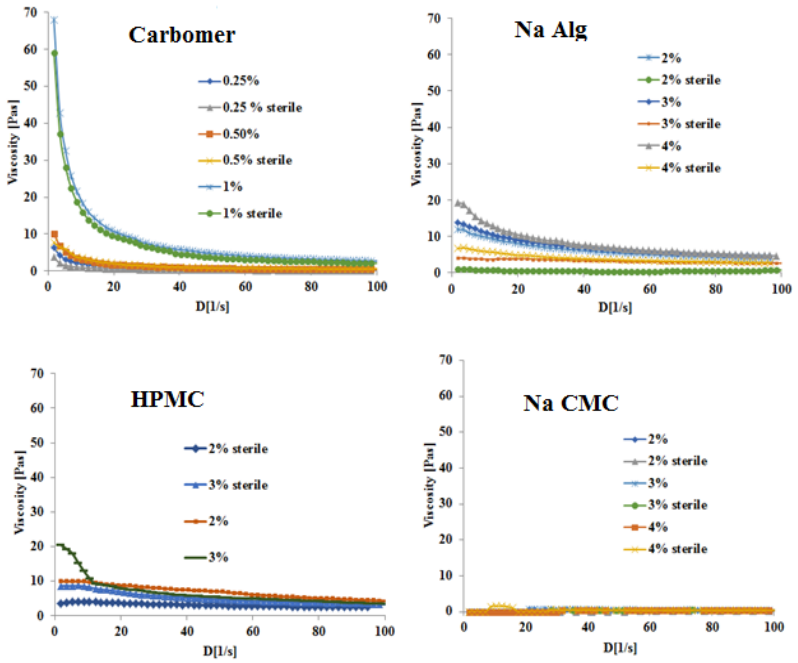


Figure 1. Plots of viscosity against shear rate in different samples.

Table 3. Apparent viscosity, n and K of prepared hydrophilic gels containing solubility enhancer

| Polymer type | Concentration (%) | Solubility enhancer type/ concentration (%) | Apparent viscosity (Pa.s) | n | K(Pa.s) |
|---|---|---|---|---|---|
| Carbomer | 0.5 | PG 1 | 18.15± 0.90 | 0.30± 0.00 | 31.70±0.02 |
| | | PG 10 | 20.10± 0.85 | 0.24± 0.00 | 37.78± 2.01 |
| | | Tween 80 0.1 | 15.25± 0.02 | 0.30± 0.00 | 22.70± 0.23 |
| | | Tween 80 1 | 3.42± 0.03 | 0.24± 0.01 | 9.56± 2.28 |
| | 0.5, sterile | PG 1 | 16.22± 0.08 | 0.31± 0.00 | 26.10 ±0.00 |
| | | PG 10 | 18.13± 0.10 | 0.29± 0.00 | 34.40 ±0.24 |
| | | Tween 80 0.1 | 15.28± 0.21 | 0.32± 0.00 | 22.85± 0.00 |
| | | Tween 80 1 | 2.23± 0.03 | 0.41± 0.00 | 5.82± 0.02 |
| Na Alg | 4 | PG 1 | 22.35±0.42 | 0.55±0.03 | 39.05±5.62 |
| | | PG 10 | 24.4 6±0.60 | 0.56±0.00 | 44.61±0.00 |
| | | Tween 80 0.1 | 23.87±0.37 | 0.54±0.00 | 41.54±0.00 |
| | | Tween 80 1 | 14.52±0.33 | 0.59±0.03 | 33.63±3.74 |
| | 4, sterile | PG 1 | 6.42±0.09 | 0.68±0.00 | 26.32±0.00 |
| | | PG 10 | 13.64±0.16 | 0.60±0.00 | 28.54±0.00 |
| | | Tween 80 0.1 | 6.18±0.02 | 0.78±0.02 | 11.28±2.82 |
| | | Tween 80 1 | 4.49±0.07 | 0.78±0.00 | 8.34±0.00 |
| HPMC | 3 | PG 1 | 21.88±0.11 | 0.56±0.01 | 33.15±1.52 |
| | | PG 10 | 25.70±0.14 | 0.51±0.00 | 45.80±0.00 |
| | | Tween 80 0.1 | 21.05±0.12 | 0.59±0.00 | 29.32±0.00 |
| | | Tween 80 1 | 16.32±0.03 | 0.74±0.00 | 13.24±0.00 |
| | 3, sterile | PG 1 | 8.67±0.09 | 0.57±0.00 | 22.01±0.00 |
| | | PG 10 | 9.63±0.00 | 0.55±0.00 | 25.95±0.00 |
| | | Tween 80 0.1 | 6.9± 0.00 | 0.62± 0.00 | 17.97± 0.00 |
| | | Tween 80 1 | 3.51±0.03 | 0.68± 0.01 | 12.89± 0.49 |

used as proper hydrophilic gel in the rest of study.

### *3.2. Rheological measurements of selected hydrophilic gels containing solubility enhancers before and after sterilization*

Tween 80 and PG were added to selected hydrophilic gels and all data are shown in Table 3 and figure 2.

As it is obvious, presence of Tween 80 at higher concentration resulted in lower viscosity. However, higher concentrations of PG increased the viscosity ($p<0.05$). P.G molecules have high ratios of hydroxyl functional groups that would form tightly orientated hydrophilic gels. In contrast, Tween 80 could decrease these hydrogen bonds and reduce the viscosity (21, 22).

Both solubility enhancers at lower concentrations did not have significant effect on the viscosity of hydrophilic gels ($P> 0.05$). The viscosity of Na Alg samples was decreased significantly after the addition of PG and Tween 80 at all concentrations and these samples were excluded from the study. As a result, carbomer 0.5 % and HPMC 3% containing Tween 80 0.1% and PG 1% were selected as proper hydrophilic gel bases that showed better resistance to moist heat sterilization and fewer viscosity changes.

### *3.3. Content uniformity of sample drug*

#### *3.3.1. Calibration Curve validation*

As it was mentioned in section 2.7, content uniformity of gels containing ATR 1% was evaluated by analysis method. Calibration curve data was constructed in the range of 2 to 10 µg/mL. Correlation coefficient (r2) of the standard curve (0.9987) indicated linear relationship at se-

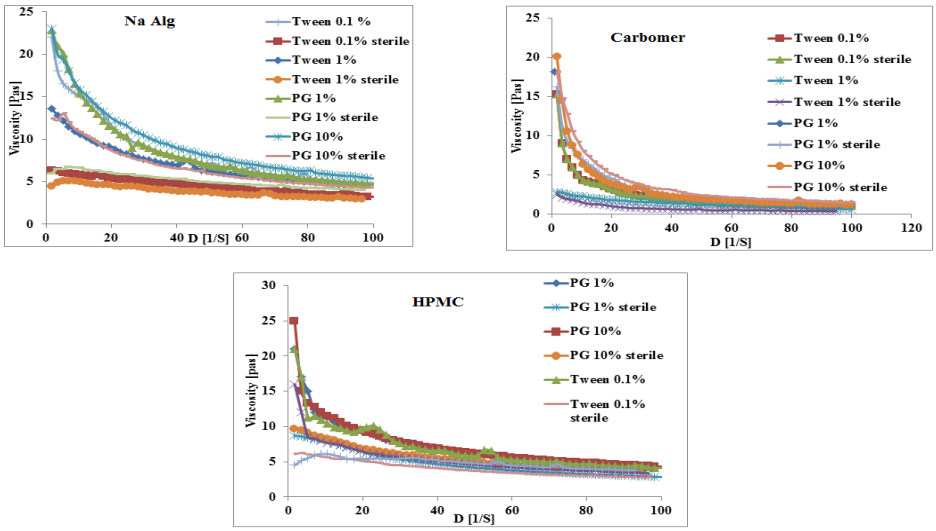


Figure 2. Plots of viscosity against shear rate in selected samples containing solubility enhancers.

lected range of ATR concentrations. Precision, accuracy, LOD and LOQ are illustrated in Table 4, which are in acceptable ranges.

### *3.3.2. Content uniformity of bases*

ATR content of gels was examined by ATR analysis method and data are shown in table 5.

As it is seen, content uniformity of HPMC base decreased after autoclaving. This would be because of HPMC behavior during sterilization process. HPMC undergoes reversible sol to gel transformation upon heating. Depending on the concentration of HPMC, its gelation temperature is 50–90°C. Beyond the gelation temperature, viscosity increases as temperature increased and it clots at sterilization process (18). As a result, content uniformity of the solubilized drug decreases. Based on the content uniformity data, HPMC gel was excluded from the rest of study.

### *3.4. Rheological behavior of hydrophilic gels containing ATR*

As it is obvious in table 6, viscosity increased in all samples in comparison to base gel after addition of ATR to gel base and viscosity was increased. This could be because of some insoluble particles of ATR in gel base that enhanced the viscosity.

Table 4. Calibration Curve Validation Containing Different Concentrations of ATR.

| Equation | r2 | Precision Intraday% | Precision Interday% | Accuracy% | LOD (µg/mL) | LOQ (µg/mL) |
|---|---|---|---|---|---|---|
| y=0.0798x+0.031 | 0.9987 | 97.93± 1.56 | 96.69± 2.36 | 99.76± 3.09 | 0.59 | 1.79 |

## 4. Conclusion

In this paper, the viscosity of different polymeric gels (in various concentrations at steam sterilization temperature), and the influences of solubility enhancers and drug presence were determined. Data showed that carbomer and HPMC

Table 5. ATR content in gels.

| Sample | Solubility Enhancer | Content (%) | |
|---|---|---|---|
| | | sterile | Non sterile |
| Carbomer 0.5 % | PG 1% | 101.2± 1.5 | 100.7± 0.7 |
| | Tween 80 0.1 % | 99.5± 0.8 | 101.0± 0.6 |
| HPMC 3% | PG 1% | 92.4±6.8 | 101.4±1.4 |
| | Tween 80 0.1 % | 93.3±8.3 | 100.3±0.9 |

Table 6. Apparent viscosity, n and K of prepared carbomer gel containing ATR.

| Polymer type | Sterility property | Solubility enhancer type and concentration (%) | Apparent viscosity (Pa.s) | n | K (Pa.s) |
|---|---|---|---|---|---|
| Carbomer 0.5 % | Non sterile | PG 1 | 19.34±0.11 | 0.10± 0.00 | 35.82±0.02 |
| | | Tween 80 0.1 | 15.66±0.09 | 0.15± 0.01 | 27.36± 1.50 |
| | sterile | PG 1 | 17.00±0.14 | 0.12± 0.00 | 30.50±0.61 |
| | | Tween 80 0.1 | 16.48±0.17 | 0.13± 0.00 | 27.51± 0.02 |

could withstand steam sterilization better than Na Alg and Na CMC without changing their viscosity. In addition, presence of solubility enhancers such as PG and tween 80 and a sample drug would affect the viscosity of the samples based on their concentrations. Carbomer 0.5% showed the best results after autoclaving and adding ATR and would be a proper topical drug delivery system to damaged areas of skin. Further studies are required to investigate other gelling properties of polymers and drug samples.

## Conflict of Interest

None declared.

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