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RESEARCH PAPER

Effect of Buffers on the Properties of Microbicidal Hydrogels Containing Monoglyceride as the Active Ingredient

T. Kristmundsdóttir,[1,*] P. Sigurdsson,[1] and H. Thormar[2]

[1]Faculty of Pharmacy and [2]Institute of Biology, University of Iceland, Reykjavík, Iceland

ABSTRACT

Hydrogel formulations containing the monoglyceride monocaprin have shown potent microbicidal activity against several sexually transmitted viruses and bacteria. It is recommended that formulations for preventing infection in the vagina have a low pH as the HIV virus is inactivated at low pH. The object of the work was to investigate how incorporation of buffers into the hydrogel formulations affects physicochemical properties and microbicidal activity of the active substance. Two series of gels were formulated using carbomer (Carbopol 934) and sodium carboxymethylcellulose (NaCMC) as gel-forming agents. The presence of buffers in the gels caused a lowering in gel viscosity, with carbomer gels being more sensitive to buffer presence than NaCMC gels. To obtain viscosity similar to that of a gel without buffer, the amount of polymer needs to be increased. An increase in the amount of NaCMC by 60–70% is needed to obtain the same viscosity as in gel without buffers; but for carbomer, the amount of polymer needs to be doubled. It appears that the effect of maleate buffer on NaCMC gel formation is greater than that of the citrate/lactate buffer; but for carbopol gels, the effects of the buffer systems tested on gel viscosity were equal. The virucidal activity of NaCMC gel buffered with citrate/lactate buffer against herpes simplex virus type 1 and HIV was not reduced by the presence of buffer. The results show that the presence of buffers in the hydrogel formulations affects gel viscosity, but the virucidal effect of the active compound, monocaprin, is not diminished.

Key Words: Monocaprin; Hydrogel; Buffers; Carbomer; Sodium carboxymethylcellulose.

*Correspondence: T. Kristmundsdóttir, Faculty of Pharmacy, University of Iceland, Hagi, Hofsvallagata 53, IS-107 Reykjavík, Iceland; Fax: 354-525-4071; E-mail: thordisk@hi.is.

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INTRODUCTION

Several fatty acids and 1-monoglycerides have been found to kill enveloped viruses and bacteria. It has been suggested that they may be used as intravaginal microbicides for protection against sexually transmitted diseases.[1,2] Hydrogel formulations containing the compound that showed the most virucidal activity, monocaprin, were found to possess potent in vitro microbicidal activity against human immunodeficiency virus type 1 (HIV-1), herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), *Chlamydia trachomatis* and *Neisseria gonorrheae* .[3,4] In mice, intravaginal infection with HSV-2 and the associated mortality were prevented completely when the infection was carried out in the presence of the monocaprin containing gel formulation.[5] The monocaprin hydrogels were formulated using as carriers mucoadhesive polymers commonly used for drug delivery, sodium carboxymethylcellulose (NaCMC) and carbomer. Mucoadhesive polymers ensure a large area of contact with the surface and prolong residence time, thereby optimizing availability of the active compound.[6–9]

It is recommended that formulations for preventing infection in the vagina have a low pH as the HIV virus is inactivated at low pH.[10,11] The pH of vaginal fluid ranges from 3.7 to 6.3, with an average of 4.9.[12] As semen is mixed with the vaginal fluid, the pH in the vagina increases to 7.0–8.0, which is the ideal condition for HIV infection.[10] In vitro work suggests that maintenance of an acid environment may inhibit HIV activity.[13] It would therefore appear preferable to include buffers in hydrogel formulations, intended for protection against infection, to keep the pH in the surrounding medium around pH 5.

The aim of the present study was to investigate the effect of buffers on physicochemical properties, as well as microbicidal effectiveness of the monocaprin containing hydrogel formulations. Previous work has shown that the gel-forming polymers, NaCMC and carbopol, are sensitive to pH and ionic strength. Therefore, the presence of buffers could affect the gel properties.

MATERIALS AND METHODS

Materials

Monocaprin, NaCMC (viscosity of 1% aqueous solution, 1,500–3,000 cps at 25°C), and polyvinylpyrrolidone (PVP, average molecular weight 40,000) were purchased from Sigma Chemical Co. (St. Louis, MO). Carbomer (Carbopol 934P) was obtained from Nomeco (Copenhagen, Denmark), and hydroxypropylmethylcellulose (HPMC; average molecular weight 4,000) was from Aldrich Chemical Company, Inc. (Milwaukee, WI). All other chemicals were of reagent grade.

Hydrogel Formulations

Hydrogels containing 20 mM monocaprin were formulated using as gel-forming agents either NaCMC and PVP (series 1) or carbomer and HPMC (series 2).

Formulations in series 1 are based on 2% w/v NaCMC and 1% w/v PVP. Gel 1 is produced by adding 1.0 g NaCMC and 0.5 g PVP to a solution of monocaprin in glycofurol 75. The pH is adjusted to 5.0 by the dropwise addition of a lactic acid solution. Finally, purified water is added, bringing the solution to a final weight of 50 g. Immediately after the addition of water, the solution is stirred continuously until a hydrogel is produced. The hydrogel is centrifuged at high speed (> 8,000 rpm) for 60 min to remove air bubbles. Gels 1C/L, 1L, and 1M are produced in the same way except the pH is adjusted to 5.0 by the addition of citrate/lactate, lactate, or maleate buffers, respectively. The buffers used were citrate/lactate buffer pH 5.0 (0.6 M trisodium citrate and 0.7 M lactic acid), lactate buffer (0.121 M lactic acid and NaOH added to pH 5.0), and maleate buffer (0.6 M maleic acid and NaOH added to pH 5.0). Five milliliters of buffer were used for the production of 50 g hydrogel.

Formulations in series 2 are based on 0.5% w/v carbomer and 1% w/v HPMC. Gel 2 is produced by dispersing 0.5 g HPMC in 10 mL of hot (80°–90°C), purified water in a glass beaker. The solution is allowed to cool to about 30°–35°C under continuous stirring at room temperature and then chilled in a refrigerator at about 4°C for at least 1 hr. Carbomer (0.25 g) is suspended in 5 mL of purified water at room temperature under vigorous stirring to prevent lumping. The carbomer solution is mixed with the HPMC solution and then a solution of monocaprin is added. Gelling of the carbomer polymer is induced by raising the pH to approximately 5.0, with the dropwise addition of a 2% w/v sodium hydroxide solution. The hydrogel is brought to its final weight (50 g) with purified

water. Finally, the hydrogel is centrifuged at high speed (> 8,000 rpm) for 60 min to remove air bubbles. Gels 2C, 2C/L, and 2M are produced in the same way, except the pH is adjusted to 5.0 by the addition of citrate, citrate/lactate, or maleate buffers, respectively. Five milliliters of buffer were used for the production of 50 g hydrogel.

HPLC Assay of Monocaprin

The monoglyceride content was determined using a high-performance liquid chromatography (HPLC) component system consisting of a Thermo Separations Products Spectra Series P200 HPLC solvent delivery system, a μBondapak^{TM} C_{18} 125A 10 μm (3.9 × 300 mm) column, a Waters Intelligent Sample Processor (WISP^{TM}) model 710B, a Thermo Separations Products SP4400 Integrator, and a Thermo Separations Products Spectra Series UV150 detector. The wavelength was 218 nm, and the mobile phase consisted of acetonitrile, water, and tetrahydrofuran (57:42:1), with the retention time being 2.1 min at 1.25 mL/min flow rate.

Drug Release from Hydrogels

Release of monoglyceride was investigated using a membraneless diffusion cell at 32 ± 0.5°C. A petri dish (41.2 mm in diameter) was filled with 3 g of gel, paced at the bottom of a dissolution vessel, and a stainless-steel support disk (Millipore Corp., Massachusetts, USA) positioned on top of the petri disk to hold the assembly at the bottom of the vessel. A paddle was placed so that a distance of 25 ± 2 mm was between the bottom of the paddle blade and the surface of the gel. One hundred milliliters of receiver phase were added and stirred at 100 rpm. The receiver phase contained 0.1% of 2-hydroxypropyl-β-cyclodextrin, and the pH was adjusted to pH 4, 5, or 6 by the addition of 51 mM of lactic acid and NaOH added to the desired pH.

Five-milliliter samples were taken from the receiver phase at regular intervals and filtered through a 0.22-μm membrane filter. After each sampling, the volume was replaced. The amount of monoglyceride released was determined by HPLC using a calibration curve of the monoglyceride in the receiver phase. Each experiment was carried out in triplicate.

Viscosity and pH Measurements

A Brookfield Digital Viscometer (model DV-II, Brookfield Engineering Laboratories, Stoughton, MA), with a small sample adapter (model SSA 25/13R) and a SC4-25/13R spindle, was used to measure the viscosity of the gels in cps. The temperature of the sample was adjusted to 25°C. Each experiment was carried out in triplicate.

pH was measured using an Orion 520A pH-meter. When determining the buffer capacity of the hydrogel formulations, 50 or 100 μL of 0.5 M NaOH solution were added to 3.33 g of gel and the pH measured. Each experiment was carried out in triplicate.

Virus and Virus Titration

Herpes simplex virus type 1 strain MacIntyre (from the American Type Culture Collection, Rockville, MD) was tested in monolayers of CV-1 cells grown in Dulbecco's Modified Eagle Medium (D-MEM) with 10% fetal bovine serum (FBS) and maintained in D-MEM with 2% FBS. Virus stocks with an infectivity titer of $10^{6.5}$–$10^{7.5}$ $CCID_{50}$ (50% cell culture infective dose) per 100 μL were used in the experiments. Virus was titrated by inoculation of 10-fold dilutions in maintenance medium into monolayers of CV-1 cells in 96-well microtiter tissue culture plates (Nunc, Roskilde, Denmark). One hundred microliters of each virus dilution were inoculated into quadruplicate wells. The plates were incubated at 37°C in a humidified incubator with 5% CO_2 in air and examined for cytopathic effect daily for 5 days.

HIV-1 strain IIIB was obtained from persistently infected H9 cells and was provided by R. C. Gallo, when at the National Cancer Institute (Bethesda, MD).[14] HIV-1 strain IIIB was tested in MT-4 cells, which were grown and maintained in RPMI 1640 medium with 10% FBS.

Assay of Virucidal Activity of Hydrogel Formulations

A hydrogel (200 μL) was placed in a 35 mm tissue culture dish (Nunc, Naperville, IL), and an equal volume of HSV-1 in medium was added. The hydrogel was thoroughly mixed with the virus at room temperature for 1, 5, or 10 min. The mixtures were then immediately diluted 100-fold in medium and titrated in 10-fold dilutions. Virus mixed with

medium instead of hydrogel was used as a control. Virucidal activity against HSV-1 in semen was tested in the same way, except that the virus was first concentrated 10-fold by centrifugation in a Sorvall ultracentrifuge at $100{,}000 \times g$ for 90 min and then diluted 1:10 in fresh (<2 hr), liquefied human semen. Virus-spiked semen mixed with medium was used as a control.

Human immunodeficiency virus type 1 diluted 1:10 in human semen was similarly mixed with hydrogels for a given time and assayed in 5-fold dilutions in microtiter plates with MT-4 cells. The titers were compared with controls to evaluate inactivation.

RESULTS AND DISCUSSION

Effect of Buffer Type on Gel Viscosity

The pH of all the gel formulations was adjusted to pH 5 using either lactic acid (NaCMC gel 1), NaOH (carbomer gel 2), or a buffer system. The buffer systems used for adjusting the pH to 5 were lactate buffer, citrate/lactate buffer, and maleate buffer. In preliminary experiments, citrate buffer was used in the formulations, but because it affected the gel formation of both polymers severely, it was decided to abandon its use.

If the buffer was added before swelling of the polymer, the gel structure was affected more than if the buffer was added later on in the gel production. Figure 1 shows the viscosity of six hydrogel formulations. The viscosities of the two hydrogel formulations without the buffers are equal. The presence of buffers in the gels causes a decrease in viscosity in all formulations, and the timing of the buffer addition to the formulations was of importance. The citrate/lactate buffer appeared to prevent gel formation of both polymers if added before gel formation (hydration of the polymer), but the effect was less pronounced if the buffer was added at the final stage of preparation. The effect of the lactate buffer on gel viscosity is not as evident as that of the citric/lactate buffer. The largest decrease in viscosity was caused by citrate/lactate buffer on the carbomer gel, but the carbomer gel appears to be more sensitive to the effect of buffers than the NaCMC gel.

To obtain similar viscosity for carbomer gels containing either citrate/lactate buffer or maleate buffer to that of gels without buffer, it is necessary to double the amount of carbopol from 0.5% to 1.0% (Fig. 2). Figure 3 compares the effect of NaCMC quantity on the viscosity of gels with and without buffer. When a buffer is included in the formulation, an increase in the amount of gel-forming agent by 60–70% is needed to obtain gel viscosity equal to that of a gel without buffers. It appears that the effect of maleate buffer on the NaCMC gel formation is greater than of the citrate/lactate buffer; but for carbomer gels, the effect of the two buffer systems on gel viscosity was equal.

In the presence of ions, the charged carboxyl groups are shielded. Thus, the polymer adapts a less expanded structure resulting in a change in the rheological

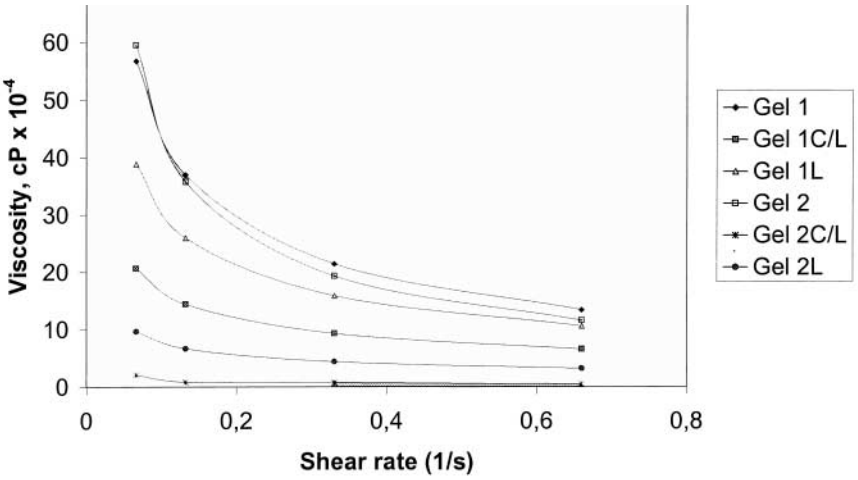

Figure 1. Viscosity as a function of shear rate for six hydrogel formulations, gel 1 (NaCMC) and gel 2 (carbomer) with citrate/lactate buffer (C/L) or lactate buffer (L).

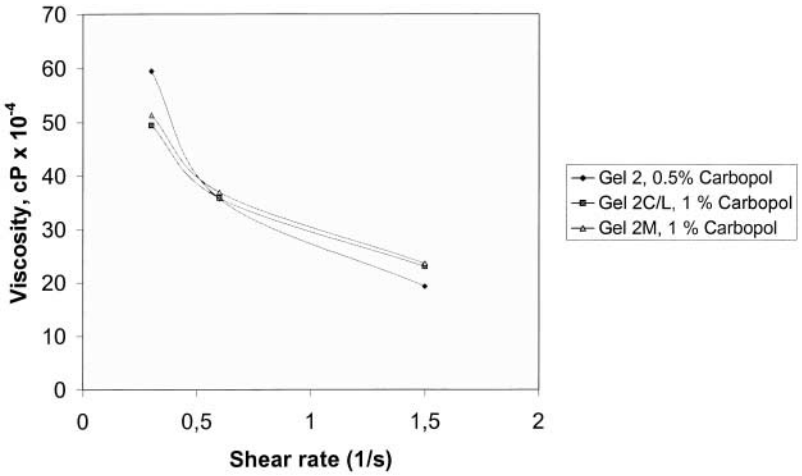

Figure 2. Viscosity as a function of shear rate for gel 2 (0.5% carbomer) and gels containing 1% carbomer and citrate/lactate buffer (2C/L) or maleate buffer (2M).

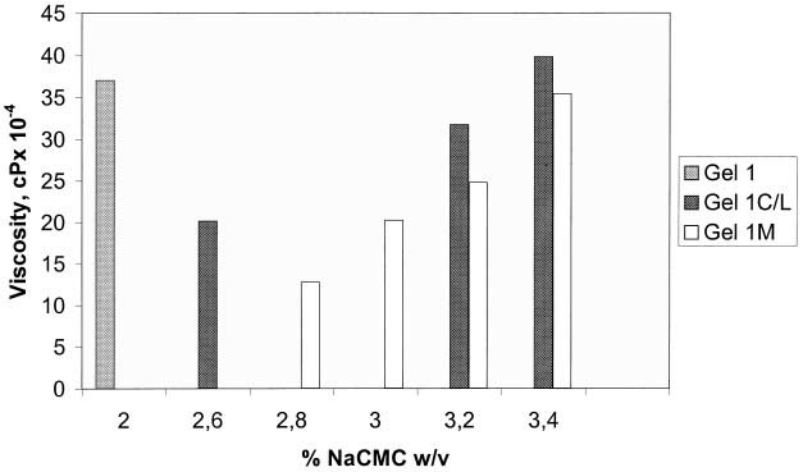

Figure 3. Viscosity of NaCMC gels containing citrate/lactate buffer (gel 1C/L) or maleate buffer (gel 1M).

properties. Higher concentration of polymer is therefore needed to obtain contact between the particles.

The lowering in gel viscosity caused by the presence of buffers is greater than could be acceptable in a commercial product. This lowering of viscosity could, however, be offset by increasing the polymer concentration.

Comparison of Buffer Capacity of Gel Formulations

Figure 4 compares the buffer capacity of the various hydrogel formulations by showing the change in gel pH with the addition of a NaOH solution. Comparison was made with two commercial vaginal gels: Aci-Jel® and Gynol-Plus®. The results show that the commercial preparations Aci-Jel and Gynol-Plus have higher buffer capacity than gels 1 or 2. There is little difference between formulations 1 and 2, but the addition of a large amount of NaOH has a more pronounced effect on the pH of gel 1. The lactate buffer showed very limited buffer capacity in the pH range examined. Maleate buffer containing hydrogels has a similar buffer capacity as the commercial preparations. The buffer capacity of the gels containing the citrate/lactate buffer, 1C/L and 2C/L, is higher than that of the commercial

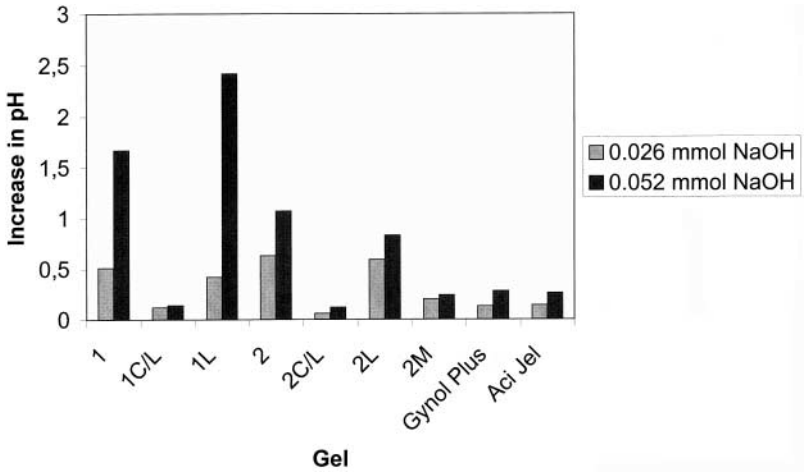

Figure 4. Effect of the addition of NaOH on the pH of hydrogel formulations.

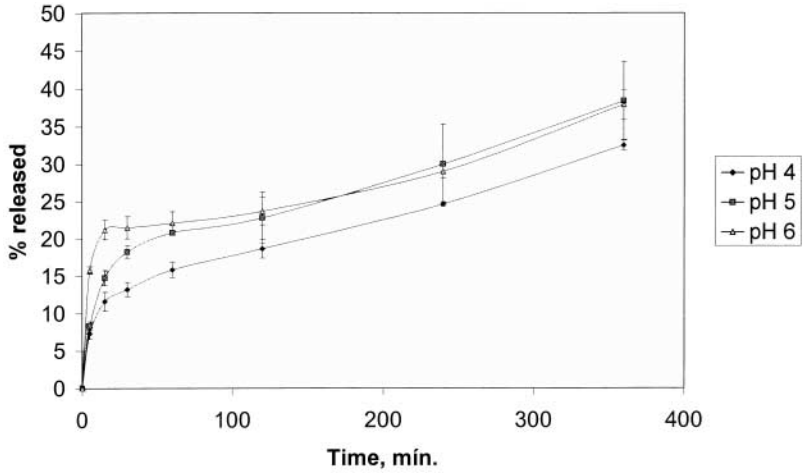

Figure 5. Effect of receiver phase pH on monocaprin release from gel 1C/L.

preparations, and gel 2C/L is better than gel 1C/L. Although the carbomer gels are more sensitive to the viscosity-lowering effect of buffers than the NaCMC gels, the gel formulation 2C/L appears to be a more useful choice because of its higher buffer capacity.

In Vitro Study of Drug Release: Effect of Receiver Fluid pH on Monocaprin Release

Drug release profile from the series 1 hydrogels, containing NaCMC as the gelling agent, show a burst-effect phenomenon during the first 30 min. The second part of the release curves show a quasi-zero-order release of monocaprin.

The drug release profiles from gel 1 C/L, NaCMC (containing 3.3% polymer; i.e., 65% increase in polymer compared with gel without buffer) hydrogels containing citrate/lactate buffer are shown in Fig. 5. The pH of the receiver fluid has an effect on the release rate, with the slowest release being into fluid with pH 4. The largest burst release of monocaprin is into medium with pH 6. After 15 min, the release rate decreases; but, as shown in Fig. 6, the pH of the receiver fluid is low-

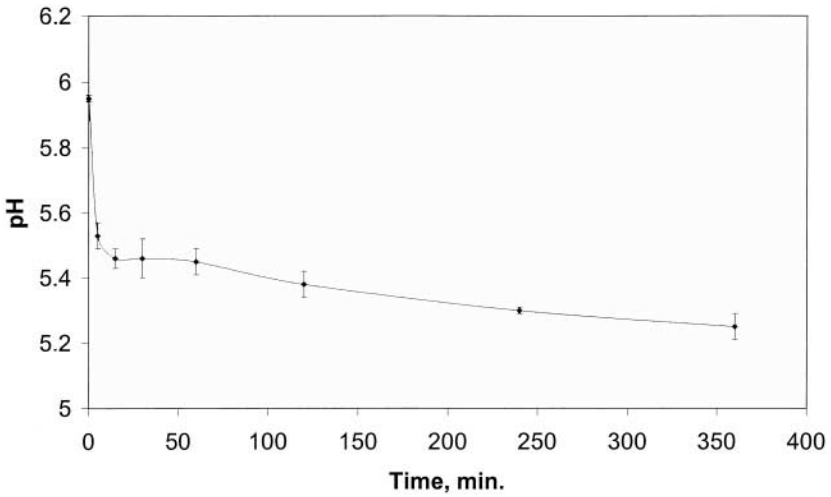

Figure 6. Change in receiver phase pH during monocaprin release from hydrogel 1C/L.

Table 1. Inactivation of HSV-1 diluted 10-fold in human semen and incubated with an equal volume of gel formulations.

Gel	Monocaprin concentration[a] (mM)	Incubation (min)	Reduction of virus titer[b] Log_{10}
1	0	10	0.7
	20	1	> 5.5
1C/L	0	10	0.3
	20	1	> 5.5
1M	0	10	0.3
	20	1	> 5.5
2	0	10	0.5
	20	1	> 5.5
2C/L	0	10	0
	20	1	4.5
2M	0	10	0.3
	20	1	5.0

[a]Reduced by half in the final mixtures.
[b]Data are the means for three experiments.

ered from the original pH 6 to pH 5.5 within 5 min of the start of the experiment. The pH of the receiver fluid with pH 4 or 5 was virtually unchanged during the experiment, but the receiver fluid with pH 6 in the beginning was changed to pH 5.15 at the end of the experiment (after 6 hr). The swelling characteristics of the gel-forming agents depend on pH and ionic strength, and the swelling increases with increasing pH of the surrounding medium.

Virucidal Effect of Formulations

Previous work had shown that the effectiveness of monocaprin-containing hydrogels on viruses can be affected by the presence of excipients.[3] Table 1 shows the inactivation of HSV-1 by gels containing 20 mM of monocaprin. With the exception of gel formulation 2C/L, the buffered formulations gave results comparable with those achieved by unbuffered

formulations 1 and 2—i.e., inactivated the virus ≥100,000-fold (>5 log) in 1 min. Formulation 2C/L caused a 50,000-fold reduction in virus titer. The control gels caused a ≤10-fold reduction in titer.

The effectiveness of gel formulation 1C/L against HIV-1 was tested, and the results were comparable with those obtained previously for unbuffered formulations 1 and 2.[4] The gels with 20 mM of monocaprin caused a greater than 10,000-fold reduction in 2 min, gel 1 caused a ≥4.3 log reduction, and gel 1C/L caused a ≥3.9 log reduction in virus titer.

The results show that the virucidal effect of monoglyceride is not diminished by the presence of the buffer.

CONCLUSIONS

The addition of buffer to the hydrogel formulations affect gel viscosity; and to obtain viscosity similar to that of gel without buffer, the amount of polymer needs to be increased. The NaCMC gel is not as sensitive to the effect of buffers on viscosity as the carbomer gel. An increase in the amount of NaCMC by 60–70% is needed to obtain the same viscosity as a gel without buffers, whereas the amount of carbomer needs to be doubled. It appears that the effect of maleate buffer on the NaCMC gel formation is greater than of the citrate/lactate buffer; but for carbomer gels, the effect of the buffer systems on gel viscosity was equal. Of the buffers used, the citric/lactate buffer proved to have the highest buffer capacity. The presence of buffers in the formulations affects gel viscosity, but the virucidal effect of the active compound, monocaprin, is not diminished.

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