AAPS PharmSciTech **2003; 4 (3) Article 30 (http://www.pharmscitech.org).**

Coprecipitation of Nonoxynol-9 With Polyvinylpyrrolidone To Decrease Vaginal Irritation Potential While Maintaining Spermicidal Potency

Submitted: January 1, 2003; Accepted: June 6, 2003

Philip T. Fowler,[1] Gustavo F. Doncel,[2] Paul M. Bummer,[1] and George A. Digenis[1]

[1]Division of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082
[2]CONRAD Program, Department of Obstetrics and Gynecology, Eastern Virginia, Medical School, Norfolk, VA

ABSTRACT

The aim of this study was to test the hypothesis that polyvinylpyrrolidone (PVP) would increase the critical micelle concentration (CMC) of nonoxynol-9 (N-9), providing a reduction in its irritation potential, while maintaining essential spermicidal activity. Solid coprecipitates of N-9 with PVP were manufactured with the use of a modified lyophilization process. The irritation potential of N-9 was estimated by an in vitro assay, monitoring the extent of hemolysis of red blood cells. CMCs of N-9 were measured in the presence of various concentrations of PVP. A modified Sander-Cramer assay was implemented to measure the spermicidal activity of N-9 and the N-9/PVP coprecipitates. With the use of the lyophilization process and more suitable solvents, solid coprecipitates of N-9/PVP were manufactured with no residual organic solvents. The irritation potential of N-9 was reduced when in the presence of PVP–50% hemolysis values increased from 0.054mM to more than 0.2mM. N-9 CMC values increased in the presence of PVP from 0.085mM (0% PVP) to 0.110mM (3.5% PVP) and 0.166mM (10% PVP). However, spermicidal activities ranged from 0.213mM to 0.238mM, N-9 remaining steady regardless of the amount of PVP. By use of N-9/PVP coprecipitates, the self-association properties and irritation potentials of N-9 were altered. This result suggests a process to produce a spermicidal product that reduces the detrimental implications to the vaginal epithelium while maintaining the essential spermicidal activity.

KEYWORDS: micelle, HIV, red blood cell assay, hemolysis, Sander-Cramer assay

Corresponding Author: George A. Digenis, Division of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082. Phone: (859) 257-1970; Fax: (859) 257-7585; Email: digenis@pop.uky.edu.

INTRODUCTION

Recently it has been reported that the prevalence of sexually transmitted diseases (STDs) is increasing at an alarming rate. It has been reported that over 300 million people worldwide are currently infected with an STD.[1] This pandemic affects not only less developed countries but developed nations as well. In the United States, approximately 12 million people are newly infected each year with an STD-causative pathogen.[2] The rate of heterosexual transmission of pathogens has dramatically grown and is now the leading category of infections. In order to inhibit the continued growth of infection rates, it is imperative that women have access to pharmaceutical products allowing control of their own protection from STD pathogens.[3] Microbicides administered vaginally to inactivate microbes at the site of transmission might address this need for women.

Research is currently under way to study the effects of various microbicides on the transmission of pathogens across the vaginal mucosa. Many of the current antimicrobial agents used in vaginal preparations are amphiphilic in nature. The majority of commercially available spermicidal formulations contain an amphiphilic compound as the active agent. Nonoxynol-9 (N-9, **Figure 1**) is the most commonly used spermicidal agent and has been reported to possess antimicrobial properties.[4] N-9 is composed of multiple oligomers that vary in ethyleneoxide chain length and in biological performance.[5,6]

Toxicity to tissues in contact with amphiphilic antimicrobial agents has become a growing area of interest. A significant drawback to the use of these agents to combat transmission of pathogens was confirmed when aggregates of certain amphiphilic compounds were demonstrated to possess toxic effects toward various tissues.[7,8] In order to increase the safety of these compounds, various formulation approaches have been employed to reduce their toxicity.[9-12] Due to its amphiphilic character, N-9 forms aggregates, known as micelles, in solution at finite concentrations and temperatures. It has been reported that N-9 can initiate irritation

and ulceration of mucosal tissues both in vitro and in vivo.[13-15] It has been speculated that micelles of N-9 may compromise the integrity of mucosal tissues, leading to increased transmission of sexually transmitted pathogens.[16]

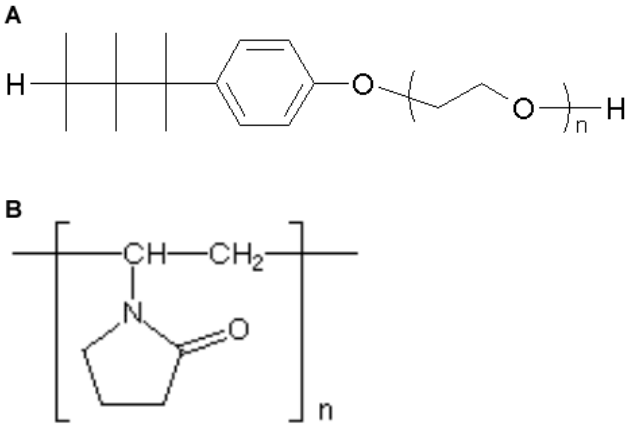

Figure 1. Structures of (A) N-9 (n = 1-17) and (B) PVP, Plasdone K/29-32.

Reducing the irritation properties of N-9 has been attempted through its formulation with various pharmaceutical polymers.[12] Polyvinylpyrrolidone (PVP, **Figure 1A**), a commonly used water-soluble polymer, has been used for many years in various pharmaceutical preparations.[17] Coprecipitates of PVP with N-9 have been produced for use in manufacturing novel spermicidal formulations as well as antimicrobial coatings for paper products.[18-20] It was theorized that the presence of PVP from the coprecipitates would decrease the interaction of the monomers of N-9, therefore decreasing the extent of aggregation of the spermicide. The decreased aggregation of N-9 molecules in solution could be evaluated by monitoring the critical micelle concentration (CMC) of N-9 in the presence of various amounts of PVP.

The aim of this study was to test the hypothesis that PVP would increase the CMC of N-9, providing a reduction in its irritation potential while maintaining essential spermicidal activity. A screening assay using red blood cells (RBCs)[10,21] was employed to assess the irritation potential of the N-9/PVP powder coprecipitates. A modified Sander-Cramer assay was used to determine if the coprecipitates altered the spermicidal activity of N-9.

MATERIALS AND METHODS

Preparation of the N-9/PVP Coprecipitate

Coprecipitation of N-9 (IGEPAL CO-630, Rhodia, Cranbury, NJ) with PVP (Plasdone K-29/32, ISP Technologies, Wayne, NJ) was accomplished by modifying previously published procedures developed in this laboratory.[18] In the previously published procedures, 1,4-dioxane was employed to facilitate proper freezing and lyophilization of the coprecipitates. In the present study, t-butanol replaced 1,4-dioxane to obtain proper freezing of the samples. Solutions of N-9 (10% wt/vol) and PVP (10% wt/vol) in methanol were placed in individual separatory funnels and allowed to drip slowly over 2 hours into a large, round-bottom flask, with continuous stirring. The volume of methanol was reduced to approximately 100 mL, under reduced pressure, followed by the addition of tertiary butyl alcohol (200 mL) to the solution. The mixture was frozen in the round-bottom flask, with continuous rotation in a liquid nitrogen bath. The alcoholic solvent was removed by freeze-drying, over a 20-hour cycle. The resulting white powder was milled to a fine powder with 3 to 4, 5-second intervals in a micromill then dried for approximately 15 hours at 75°C. The dried powder was sifted through a 100-mesh sieve and stored in a sealed polypropylene bottle.

Determination of N-9 content in PVP Coprecipitates

Triplicate samples of the N-9/PVP coprecipitate were weighed and placed into separate 10-mL volumetric flasks followed by 5 mL of methanol:water (80:20) mobile phase. Acetanilide (2 mL, 0.15 mg/mL stock) was added to the dissolved samples as an internal standard solution. Sufficient high-performance liquid chromatography (HPLC) mobile-phase solvent was then added to bring the volume to 10 mL in a volumetric flask.

A reverse-phase HPLC system (Waters Associates, Milford, MA) was used to assess the N-9 content of samples of the N-9/PVP preparations. The HPLC system consisted of a Waters analytical µ-Bondapak C_{18} column (30 cm × 3.9 mm) and a mobile phase of methanol:water (80:20). Elution was conducted at 2 mL/min at room temperature.22 Detection of N-9 in the effluent mobile phase was achieved with the use of a UV detector set at 280 nm. Prior to loading the injector, samples were filtered using a 0.45-µm cellulose acetate filter. Aliquots were injected into an analytical C_{18} column. A standard curve containing acetanilide

Table 1. Extent of Aggregation, Irritation Potential and Spermicidal Activity of Samples of N-9 in the Presence of Varying Concentrations of PVP*

Samples†	CMC Value‡ (mM)	Initial Hemolysis§ (mM)	50% Hemolysis‖ (mM)	MEC Value¶ (mM)
N-9 (0.0% PVP)	0.085 ± 0.009	0.025	0.058	0.213 ± 0.018
N-9 (3.5% PVP)	0.110 ± 0.001#	0.040	0.087	0.238 ± 0.012††
N-9 (10% PVP)	0.167 ± 0.003**	0.075	> 0.20‡‡	0.238 ± 0.012††

* CMC indicates critical micelle concentration; MEC, minimum effective concentration; N-9, nonoxynol-9; and PVP, polyvinylpyrrolidone.

† Samples of N-9 (molecular weight 617) contained 0.0%, 3.5%, and 10% PVP.

‡ CMC values were determined using a Wilhemly plate technique and were reported in mM for the concentrations of N-9 (n = 3).

§, ‖ Concentrations of N-9 at the onset of hemolysis and 50% hemolysis were determined from examination of Figure 2 (n = 3). N-9 in the presence of 10% PVP, at the concentrations tested (0.0 − 0.2mM), had a maximum percentage hemolysis of 30%.

¶ MEC values were determined using a modified Sander-Cramer assay and were reported in mM (n = 10).

# $P < .05$

** $P < .01$ versus N-9 without PVP

†† $P = 0.395$ versus N-9 without PVP

‡‡ At the maximum N-9 concentration tested (0.2mM), only 30% hemolysis was observed and a 50% hemolysis value could not be obtained.

(0.5 mg/mL) and N-9 (0.02-4 mg/mL) was made in order to determine the concentration of N-9 present in the experimental samples.

Assessment of irritation potential through hemolytic studies

The irritation potential of commercial N-9 (IGEPAL CO-630) and N-9/PVP coprecipitates was estimated using an RBC hemolysis assay.[10] Various vaginal formulations and products have been classified as topical agents.[23] The RBC hemolysis assay has been shown to be advantageous in the screening of new topical preparations for their local irritation potential.[24] In addition, irritation potentials of surfactants, such as N-9, have previously been reported using the RBC hemolysis assay.[25]

Blood samples were obtained from beagle dogs and the RBCs were isolated by centrifugation. RBCs were washed (3×) and resuspended (hematocrit level, 4%) using phosphate buffered saline (PBS, pH 7.4). Test samples were prepared by combining 0.5 mL of the RBC suspension with 3 mL of PBS containing various concentrations of N-9 and PVP from the use of the coprecipitate. Concentrations of N-9 in the experimental samples ranged from 0.0mM to 0.2mM, while the concentrations of PVP tested were 0.0%, 3.5%, and 10% PVP. Samples containing the RBCs and N-9/PVP coprecipitate were incubated at 37°C for 30 minutes, with mild shaking. Negative controls were prepared by adding PBS buffer and the appropriate concentration of PVP (no N-9 present). Positive controls were prepared by incubating RBCs in the presence of 0.2mM N-9. In addition, the samples underwent sonication and mild shaking to ensure complete hemolysis of the sample. All samples were run in triplicate (n = 3). The experimental samples were centrifuged (1500 rpm) for 3 minutes then placed in an ice bath to quench the hemolytic reaction.

Following the quenching of the hemolytic process, the absorbance of the supernatant of each sample was measured at 576 nm. The percentage of hemolysis (%H) was determined by use of the following equation:

$$\%H = 100\% \, (Abs - Abs_{control}) / (Abs_{100} - Abs_{control}) \qquad (1)$$

where Abs is the absorbance of the sample, $Abs_{control}$ is the absorbance of the control sample (negative control), and Abs_{100} is the absorbance of the sample in which 100% hemolysis occurred (positive control). For comparison of the effects of varying levels of PVP on N-9 irritation potential, the concentrations of N-9 in which initial hemolysis and 50% hemolysis occurred were compared (**Table 1**). The 50% hemolysis values were determined through the use of nonlinear regression analysis.

Assessment of Spermicidal Activity

The spermicidal effect of N-9 and the various N-9/PVP coprecipitates on human spermatozoa was assessed using a modified Sander-Cramer assay.[26] Experimental samples were prepared through serial dilutions of stock solutions containing N-9 or N-9/PVP coprecipitates in

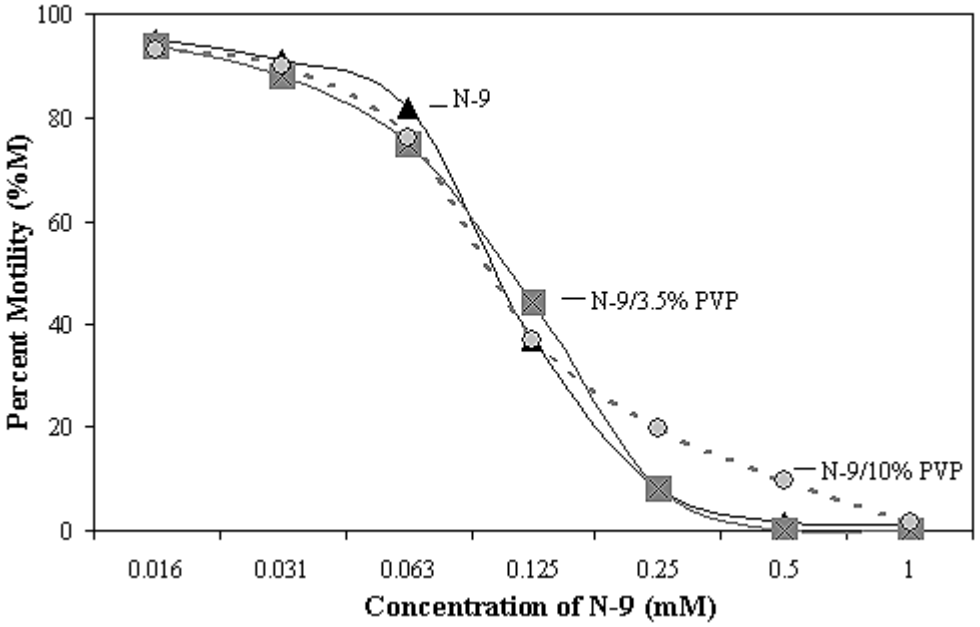

Figure 2. Spermicidal dose response curve of N-9 with varying concentrations of PVP (n = 10).

0.9% NaCl. Aliquots of these stock solutions, 250 µL in volume, were incubated (20 seconds at 25°C) with 50 µL of normal semen containing 6×10^7 motile sperm/mL. During the incubation period, the experimental samples were examined microscopically under dark-field (E600 Nikon microscope, Melville, NY) for the presence of motility. The presence of motile sperm indicated the lack of spermicidal activity, and the sample failed the terms of the assay.[26,27] Samples containing completely immobile spermatozoa were reassessed for possible reversibility of sperm immobilization. These samples were diluted with excess glucose-phosphate buffer, incubated for 60 minutes at 37°C, and then reassessed for motility under the same conditions as previously described. Using this process, a minimum effective concentration (MEC) for a sample was determined by using the highest sample dilution that induced total sperm-cell immobilization with no recovery of motility (**Table 1**). Dose response curves were constructed for N-9 (0.016mM-1mM) in the presence of various PVP concentrations by using a similar methodology (**Figure 2**). For each experimental sample, the number of motile sperm was counted then normalized versus a negative control.

Determination of Critical Micelle Concentration

Experimental solutions were prepared by dilution of a stock solution resulting in varied N-9 and PVP concentrations. The following stock solutions were prepared;0.3mM N-9 (0.0% PVP); 0.37mM N-9 with 3.5% PVP;0.41mM N-9 with 10% PVP and1.8mM N-9 with 10% PVP. The stock solutions were diluted to attain experimental concentrations of N-9 ranging from 0.00075mM to 0.72mM. Precisely 20 mL of the test solutions was added to a jacketed beaker kept at a constant temperature of 37°C. The samples were allowed to equilibrate, and the surface tension of each solution was measured using a Wilhemly plate (KSV Instruments USA, Riverside, CT). The CMC values were determined by creating a linear plot of surface tension versus concentration of N-9 (mM). From these plots, linear regression was employed in order to obtain the CMC value for N-9 at the various PVP concentrations tested (n = 3), with an average reported.

RESULTS

N-9/PVP Coprecipitates

The process to produce N-9/PVP coprecipitates was similar to those procedures previously published.[18] The resulting white, free-flowing powder was found to contain 6.16% ± 0.13% N-9 (wt/wt) using the HPLC assay described (n = 5) above.

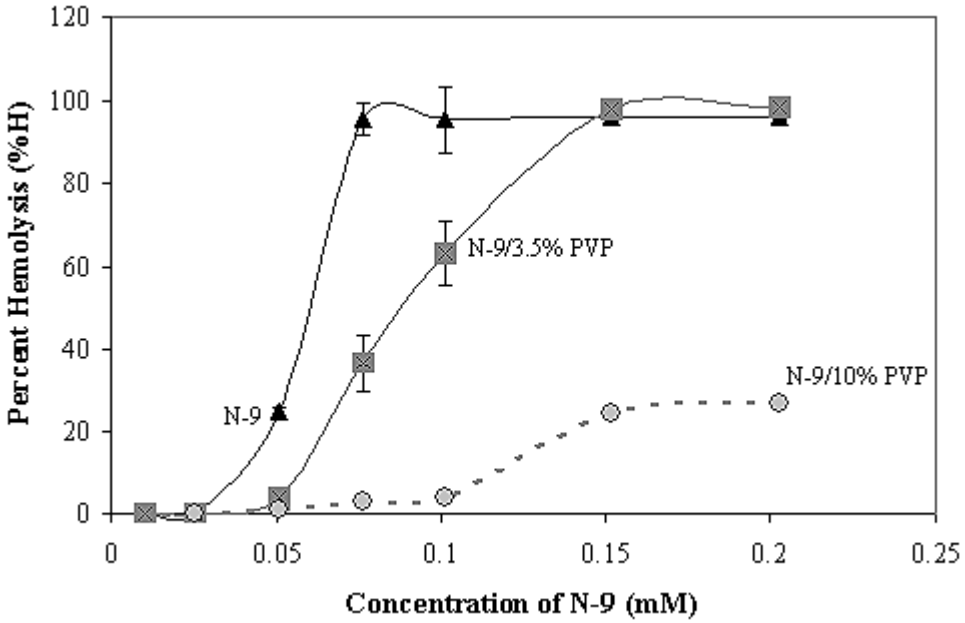

Figure 3. Measurement of percentage hemolysis (%H) of canine RBCs as an indicator of irritation potential of N-9 with varying concentrations of PVP (n = 3).

Irritation Potential as Measured by the Hemolytic Assay

Shown in **Figure 3** is the extent of hemolysis of the RBCs as a function of the concentration of N-9 in the incubation media. Inclusion of PVP in the incubation media resulted in a marked reduction in the extent of RBC hemolysis (**Table 1**, **Figure 3**). The concentrations of N-9 at which 50% hemolysis occurred were calculated using the nonlinear regression analysis software Graphpad Prism (GraphPad Software, Inc, San Diego, CA). In the absence of PVP, 50% hemolysis was observed at 0.054mM N-9, while in the presence of 3.5% PVP, 50% hemolysis was observed at 0.088mM N-9. A 50% hemolysis value could not be obtained for samples containing 10% PVP since only 30% hemolysis was detected at the highest concentration of N-9 tested.

Spermicidal Assessment with Human Spermatozoa

The results of the Sander-Cramer assay established the MEC for N-9 in the absence of PVP to be 0.213mM. In the presence of 3.5% and 10% PVP, the MEC for N-9 increased slightly to 0.238mM for both samples (**Table 1**). The differences in the results were not found to be significant when applying an analysis of variance (ANOVA) on ranks (P = .3953). By assessing the percentages of remaining motile spermatozoa after a 30-second incubation, a dose-response curve was established for N-9 in the presence and absence of PVP. In the spermicidal dose-response curve, N-9 samples possessed equivalent results independent of the concentrations of PVP employed (**Figure 2**).

Critical Micelle Concentration Values

Using the Wilhelmy plate, the CMC of N-9 in the absence of PVP was determined to be 0.085 ± 0.009mM N-9 (**Table 1**). The CMC values of N-9 in the presence of 3.5% and 10% (wt/vol) PVP were found to increase to 0.110 ± 0.001mM N-9 and 0.166 ± 0.003mM N-9, respectively (**Table 1**).

DISCUSSION

N-9/PVP Coprecipitate

The coprecipitate of N-9 contained 6.16% (wt/wt) of N-9 based on the HPLC assay. This value is only about 2% less than the amount of N-9 expected in the formulation based on the weights of the ingredients initially added. The minor difference in the N-9 content could be attributed to losses to glassware employed during the formation of the coprecipitates.

In the manufacturing procedure reported previously by Digenis et al, dioxane was used as the organic solvent in the coprecipitation of N-9 and PVP.[18] Analysis for volatile organics in the resulting powder found high levels of dioxane (25 000 ppm). Because of the reported toxicity of dioxane, the current procedure was modified to employ tertiary butyl (t-butyl) alcohol instead.[28-30] In employing t-butyl alcohol, it was found necessary prior to lyophilization to freeze the flask containing the N-9/PVP mixture in a Dewar flask filled with liquid nitrogen. This alteration to the previously published procedures resulted in powders that were free of any volatile organic compounds.

Irritation Potential as Measured by the Hemolytic Assay

In this study, PVP was coprecipitated with N-9 to minimize the irritation potential of the surfactant as assessed by the RBC screening assay. PVP with an average molecular weight of 36 000 was chosen because of its wide use in clinical medicine[17] and its optimal protective effect on RBCs against mechanical and osmotic damage.[31] The onset of hemolysis increased by increasing the concentration of PVP in the test solutions, while the extent of hemolysis was reduced when tested at constant N-9 concentrations (**Table 1**, **Figure 3**). The reduction of RBC hemolysis by PVP is possibly the result of the combination of interaction with N-9 to increase the CMC value (**Table 1**) of the surfactant and as well as the reported ability to coat the outer surface of cells yielding a mechanical barrier to damage.[17,31] The observed reduction of hemolysis of RBCs by N-9 in the presence of PVP suggests a reduction in the irritation potential of N-9. The RBC hemolysis assay employed in this study has been shown by other laboratories to be useful for assessing local irritation potential for certain preparations and their ingredients.[24] The RBC hemolysis assay was intended to be an in vitro screen for irritation potential; the true vaginal irritation assessment will need to be through an in vivo method not addressed in this study.

Spermicidal Assessment

In the studies pertaining to the spermicidal activity of N-9/PVP coprecipitates published by Zavos et al, enhancement of N-9 spermicidal activity using the coprecipitates was reported when compared with the spermicidal activity of N-9 in the absence of PVP.[32,33] The studies of Zavos et al followed the same manufacturing process that in our hands led to high levels of residual dioxane in the powders. Dioxane has been reported to exert toxic effects on nasal epithelia membranes,[28] and it is possible that the high levels of residual dioxane in the coprecipitates of Zavos et al may have led to the increase in spermicidal activity.[32] In the current study, N-9 maintained its spermicidal activity in the presence of increasing concentrations of PVP in the modified Sander-Cramer assay and dose-response study (**Table 1**, **Figure 2**). Comparing the dose-response curves, all N-9 samples had similar activities, regardless of PVP concentration, inducing a decrease in sperm motility commencing at approximately 0.063mM (**Figure 2**). At the N-9 concentration of 0.25mM, over 90% of the sperm cells were immobilized in the samples containing 0.0% and 3.5% PVP. At the same N-9 concentration of 0.25mM, the sample containing 10% PVP immobilized 80% of the sperm cells during the in vitro assay. The presence of PVP did not influence the effectiveness of N-9 to immobilize human spermatozoa.

Critical Micelle Concentration Values

The presence of PVP resulted in an increase in the concentration at which N-9 formed micelles in solution (**Table 1**). It has been reported that PVP interacts sufficiently with various nonionic surfactants in solution to change the phase behavior descriptors of cloud and Kraft points of the surfactants.[34] It is hypothesized that the interaction between N-9 and PVP would be sufficient to influence the CMC value for N-9. The literature value for the CMC of N-9 had been reported to be 0.081mM N-9.[35] In our hands, the CMC value of N-9 was determined to be 0.085mM N-9, which was judged to be in good agreement with the value reported in the literature. With the addition of 3.5% PVP, the CMC of N-9 increased by 29% to 0.11mM. At the highest concentration of PVP tested (10%), the CMC of N-9 increased over 95% to 0.166mM. Statistical significance of the experimental CMC values was assessed using a 1-way ANOVA and a paired t test. Significant differences ($P < .05$) in the CMC value of N-9 were detected among the 3 groups using different concentrations of PVP (0.0% PVP, 3.5% PVP, and 10% PVP). All experimental solutions demonstrated a reduction in surface tension at low N-9 concentrations, regardless of PVP concentration. The mechanism by which PVP increased the CMC values could not be discerned at this time.

CONCLUSION

N-9/PVP coprecipitates were formed as a function of PVP concentration, using a freeze-drying process. The

manufacturing process was altered by the substitution of dioxane as the organic solvent with t-butyl alcohol. Coprecipitates of N-9 and PVP were formed using a modified manufacturing method that implemented t-butyl alcohol as a replacement for the highly toxic dioxane used in previous methods. The CMC values of N-9 increased in aqueous solutions in the presence of PVP (3.5% and 10% PVP). Elevated CMC values suggest that the polymer interacts with the surfactant, either in the monomer state or the micelle, thereby hindering the aggregation process of N-9 in solution. In the RBC hemolysis assay, incorporating PVP in the samples decreased the onset and extent of membrane disruption of the RBCs. The reduction of hemolysis during this screening assay may be considered as an indicator that the irritation potential of N-9 was lessened in the samples that possessed increased amounts of PVP (**Table 1**, **Figure 3**). The Sander-Cramer assay and the spermicidal dose response curves both indicated that the addition of PVP did not reduce the biological activity of N-9 (**Table 1**, **Figure 2**). This study indicates that the addition of polymers, such as PVP, may decrease the self-associating properties of N-9, leading to a decrease in irritation potential without compromising spermicidal activity. If this result translated into reduced vaginal irritation, N-9/PVP coprecipitates would become promising microbicidal contraceptive candidates.

REFERENCES

1. Gerbase A, Rowley J, Mertens T. Global epidemiology of sexually transmitted diseases. Lancet. 1998;351:2-4.

2. Ebrahim S, Peterman T, Zaidi A, Kamb M. Mortality related to sexually transmitted diseases in US women - 1973 through 1992. Am J Public Health. 1997;87:938-944.

3. Elias C, Heise L. Challenges for the development of female-controlled vaginal microbicides. AIDS. 1994;8:1-9.

4. Bourinbaiar A, Lee-Huang S. Comparative in vitro study of contraceptive agents with anti-HIV activity: gramicidin, nonoxynol-9 and gossypol. Contraception. 1994;49:131-137.

5. Walter B, Hawi A, Zavos P, Digenis G. Solubilization and in vitro spermicidal assessment of nonoxynol-9 and selected fractions using rabbit spermatozoa. Pharm Res. 1991;8(3):403-408.

6. Walter B, Digenis G. High-performance liquid chromatographic (HPLC) analysis of oligomeric components of the spermicide nonoxynol-9. Pharm Res. 1991;8:409-411.

7. Barwicz J, Christian S, Gruba I. Effects of the aggregation state of amphotericin B on its toxicity to mice. Antimicrob Agents Chemother. 1992;36(10):2310-2315.

8. Yamashita K, Janout V, Bernard E, Armstrong D, Regen S. Micelle/monomer control over the membrane-disrupting properties of an amphiphilic antibiotic. J Am Chem Soc. 1995;117:6249-6253.

9. Barwicz J, Gareau R, Audet A, Morisset A, Villiard J, Gruda I. Inhibition of the interaction between lipoproteins and amphotericin B by some delivery systems. Biochim Biophys Res Comm. 1991;181(2):722-728.

10. Aramwit P, Yu B, Kwon G. The effects of serum albumin on the aggregation state and toxicity of amphotericin B. Proceed Int_l Symp Control Rel Bioact Mater. 1998;25:669-670.

11. Ladipo O, De Castro M, Filho L, et al. A new vaginal antimicrobial contraceptive formulation: phase I clinical pilot studies. Contraception. 2000;62(2):91-97.

12. Gagne N, Cormier H, Omar R, et al. Protective effect of a thermoreversible gel against the toxicity of nonoxynol-9. Sex Transm Dis. 1999;26(3):177-183.

13. Stafford M, Ward H, Flanagan A, et al. Safety study of nonoxynol-9 as a vaginal microbicide: evidence of adverse effects. J Acquir Immune Defic Syndr Hum Retrovirol. 1998;17(4):327-331.

14. Roddy RE, Cordero M, Cordero C, Fortney JA. A dosing study of nonoxynol-9 and genital irritation. Int J STD AIDS. 1993;4(3):165-170.

15. Phillips D, Taylor C, Zacharopoulos V, Maguire R. Nonoxynol-9 causes rapid exfoliation of sheets of rectal epithelium. Contraception. 2000;62(3):149-154.

16. Niruthisard S, Roddy RE, Chutivongse S. The effects of frequent nonoxynol-9 use on the vaginal and cervical mucosa. Sex Transm Dis. 1991;18:176-179.

17. Robinson B, Sullivan F, Borzelleca J, Schwartz S. PVP: A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone. Chelsea, MI: Lewis Publishers; 1990.

18. Digenis G, Nosek D, Mohammadi F, Darwazeh N, Anwar H, Zavos P. Novel vaginal controlled-delivery systems incorporating coprecipitates of nonoxynol-9. Pharm Dev Tech. 1999;4(3):421-430.

19. Digenis G, Digenis A. High-energy coprecipitates of nonoxynol-9 oligomers, PVP and iodine having contraceptive and potent anti-HIV properties. US patent 5 380 523. January 10, 1995.

20. Digenis G, Digenis A. Coated products with potent anti-HIV properties. US patent 5 492 692. February 20, 1996.

21. Elbary A, Nour S. Correlation between the spermicidal activity and the haemolytic index of certain plant saponins. Pharmazie. 1979;34(9):560-561.

22. Agha B, Garbolas G, Digenis G. Preparation of (131I) iodinated nonoxynol-9. J Labelled Comp Radiopharm. 1984;21(9):821-831.

23. Lard S, Hastings K, Feigal D. Considerations in the development of vaginal products intended to prevent the sexual transmission of HIV. Proceedings of the First Workshop on Antiviral Claims for Topical Antiseptics. May 31-June 1, 1994. Washington, DC: United States Government Printing Office; 1994. HE 20 4702.

24. Pape W, Hoppe U. In vitro methods for the assessment of primary local effects of topically applied preparations. Skin Pharmacol. 1991;4(3):205-212.

25. Pape W, Pfannenbecker U, Hoppe U. Validation of the red blood cell test system as in vitro assay for the rapid screening of irritation potential of surfactants. Mol Toxicol. 1987-88;1(4):525-536.

26. Sander F, Cramer S. A practical method for testing the spermicidal action of chemical contraceptives. Hum Fertil. 1941;6(5):134-137.

27. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. 3rd ed. New York, NY: Cambridge University Press; 1992.

28. Goldsworthy T, Monticello T, Morgan K, et al. Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes. Arch Toxicol. 1991;65(1):1-9.

29. Kasraian K, DeLuca P. Thermal analysis of the tertiary butyl alcohol-water system and its implications on freeze-drying. Pharm Res. 1995;12(4):484-490.

30. Kasraian K, DeLuca P. The effect of tertiary butyl alcohol on the resistance of the dry product layer during primary drying. Pharm Res. 1995;12(4):491-495.

31. Ben-David A, Gavendo S. The protective effect of polyvinylpyrrolidone and hydroxyethyl starch on noncryogenic injury to red blood cells. Cryobiology. 1972;9:192-197.

32. Zavos PM, Correa JR, Nosek D, Mohammadi F, Digenis GA. Assessment of new formulations of nonoxynol-9 coprecipitated with polyvinylpyrrolidone and iodine as possible vaginal contraceptives. Fertil Steril. 1996;66(5):729-733.

33. Zavos PM, Correa JR, Nosek D, Mohammadi F, Digenis GA. Comparative spermicidal performance of iodinated and non-iodinated contraceptive formulations of nonoxynol-9 co-precipitated with polyvinylpyrrolidone. Contraception. 1996;54:39-41.

34. Pandit N, Kanjia J. Phase behavior of nonionic surfactant solutions in the presence of polyvinylpyrrolidone. Int J Pharm. 1996;141:197-203.

35. Wolfinbarger, L. Process for cleaning large bone grafts and bone grafts produced thereby. US Patent 5 977 432. November 2, 1999.