Nonoxynol-9-α-Cyclodextrin Inclusion Compound and Its Application for the Controlled Release of Nonoxynol-9 Spermicide

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ABSTRACT: Nonoxynol-9 (N-9) is the most active ingredient in commercially available spermicidal products in the United States. There are many applications of cyclodextrin inclusion complexes (CD-ICs), but there are no reported studies investigating the formation of and controlled release from a N-9 spermicide-CD-IC. We have successfully formed the inclusion compound between N-9 and α-CD using a solution-heating technique. The N-9-α-CD-IC was characterized by Fourier Transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, differential scanning calorimetry, thermogravimetric analysis, and wide angle X-ray diffraction observations. Silicone elastomer (SILASTIC MDX4-4210) film embedded with crystalline N-9-α-CD-IC was prepared and evaluated for its efficacy in the controlled release of N-9 spermicide against bovine sperm. Silicone elastomer with N-9-α-CD-IC was as successful in reducing the motility and viability of bovine spermtazoa as silicone elastomer swollen with an equivalent amount of neat N-9. The permeability of the flexible silicone elastomer apparently enables the N-9 spermicide to diffuse from its embedded inclusion complex crystals to contact, immobilize, and kill bovine sperm cells. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 4104–4109, 2007

Key words: cyclodextrins; inclusion complexes; nonoxynol-9 spermicide; silicone elastomer; bovine sperm

INTRODUCTION

Cyclodextrins (CDs) are produced by glucosyltransferase enzymaric degradation of amylase starch. CDs are composed of α-(1,4)-linked D(+)-glucopyranose units and make up a family of three well-known cyclic members; α-, β-, and γ-CDs containing six, seven, and eight monosaccharides moieties, respectively. CDs have doughnut-shapes, with all the glucose units in substantially undistorted chair conformations, resulting in a unique arrangement of their —OH functional groups. The secondary hydroxyl groups are located on one side of the torus, while the primary hydroxyl groups are located on the opposite side of the torus. Thus the interior of the torus is relatively hydrophobic compared with the hydrophilic exterior, which bears all of the many hydroxyl groups. CDs have a truncated conical shape, with the secondary hydroxyl side more open than the primary hydroxyl side and with a hollow interior. Although the depth of the CD cavities is the same (7.8 Å), the size of the cavity depends upon the number of glucose units in the CD ring. The diameters of the α-, β-, and γ-CD cavities are ~ 5.7, 7.8, and 9.5 Å, respectively, (Fig. 1).[1,2]

One of the most important characteristics of CDs are their capability of forming inclusion complexes (ICs) with various compounds (guests), in which the guest compounds are included in the host CD cavities, as shown in Figure 2. CDs produce a large variety of ICs with low molecular weight and polymer guests. Their versatile capability to bind so many different guest materials is due to the nature of their internal cavities.

CD inclusion complexes are either soluble or crystalline solids. There are two different forms of ICs in the crystalline state, channel and cage-type structures. Channel structures develop when CDs stack on top of one another to yield endless channels, in which long guest molecules, like polymers (see Fig. 2), are included. Cage structures are a result of a displaced arrangement of CDs, in which entire small or parts of larger guest molecules are located in the cavities represented by the annular CD apertures.

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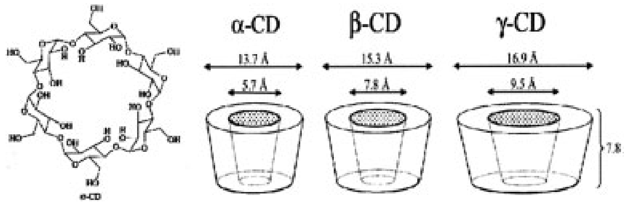

Figure 1 Cyclodextrin structure.

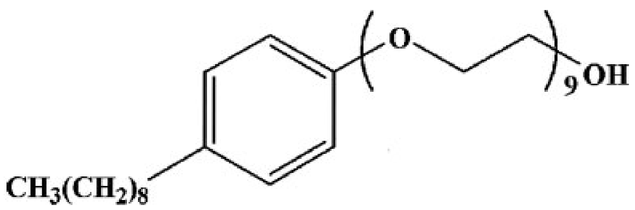

Figure 3 Chemical structure of nonoxynol-9.

There are many applications of CDs for use in pharmaceuticals, foods, cosmetics, chemical industries, as well as in agriculture and environmental engineering.[3–6] CDs can be used in drugs either for complexation or as auxiliary additives, such as carriers, diluents, solubilizers, or tablet ingredients. Their use in foods and cosmetics is for the molecular encapsulation of flavors and fragrances.

Contraception remains an important part of national efforts to reduce adolescent pregnancy. The use of condoms continues to be recommended for all sexually active adolescents to reduce the risk of acquiring sexually transmitted diseases (STDs), such as acquired immunodeficiency syndrome (AIDS). Therefore, the condom is a prominent component of strategic public health planning. Nonoxynol-9 (N-9) is a member of a category of compounds called ''nonionic surfactants'' (see Fig. 3). In the 1980s, N-9 was introduced as a supplemental method of contraception. N-9 itself has been shown to kill the active sperm in semen, thus reducing the chance of pregnancy. Therefore, it has been widely used in contraceptives for its spermicidal properties, in the forms of creams, foams, gels, films, and suppositories for over 30 years. Today it is the active ingredient in most over-the-counter spermicidal products available in the US and many other countries. It was originally thought that N-9 was an effective microbiocidal against many organisms, including HIV, even though it was never clinically proven. *In vitro* studies show that N-9 produces bacteriacidal and virucidal effects by disrupting the cell membrane and the viral envelope. But recent *in vivo* studies indicate that N-9 cannot be recommended for the prevention of HIV and other STDs. Hence, it remains unclear whether the intravaginal application of N-9 safely prevents the transmission of HIV and other STDs during sexual intercourse.[7,8]

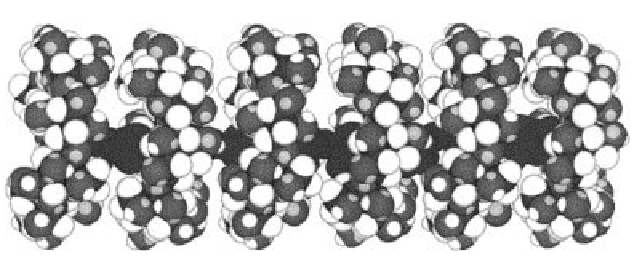

Figure 2 Inclusion complex between host (CD) and a polymer guest.

Condoms made from natural latex have dominated the market, but latex condoms have a downside. Frequent contact causes sensitivity and allergic reactions in some people. Beginning in the 1990s, polyurethane films were developed as an alternative to latex for condoms and have been approved for marketing. Although these nonlatex condoms were associated with higher rates of clinical breakage than latex condoms, the newer polyurethane condoms still provide an acceptable alternative for those with allergies, sensitivities, or preferences that might prevent the consistent use of latex condoms.[9,10] Also, silicone is rapidly becoming a preferred material due to its low initial modulus of expansion. Silicone has a higher elongation than polyurethane and a very good memory, making it the closest alternative material to latex in terms of elongation and recovery. In 1989, a Japanese patent for a condom containing spermicidal metal ions was filed. It described a condom prepared from silicone rubber containing 30 wt % Cu powder.[11] A patent for biomimetic silicone elastomers using crosslinking agents was published in 2003.[12] Also, there are many papers describing investigations of the controlled release of spermicide using polymeric devices including silicone.[13–18]

Today in the US and global markets, condoms may be packed dry or with an added lubricant, which may contain N-9 spermicide. There are also many papers dedicated to the industrial application of CD inclusion compounds. However, no studies examining the spermicidal efficacy of N-9-α-CD-IC crystals embedded into condoms have been reported. Here we examine how N-9 spermicide complexed in its α-CD-IC crystals will affect the reduction of sperm motility. When N-9-α-CD-IC is embedded into condoms, the included guest, N-9, may be slowly released, suggesting potential applications in the controlled release of spermicide. Also, concerns have been expressed that condoms lubricated or swollen with N-9 have a shorter shelf-life, while condoms embedded with N-9-α-CD-IC crystals may, on the other hand, have an extended shelf-life.

We formed the α-CD IC with N-9, and characterized the N-9-α-CD-IC using Fourier Transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and wide angle X-ray diffraction to confirm IC formation. A silicone elastomer embedded with N-9-α-CD-IC crystals was prepared and evaluated for the controlled release and spermicidal action of N-9 against previously frozen bovine sperm.

EXPERIMENTAL

Materials

Nonoxynol-9, α-CD, and Silastic medical grade elastomer (MDX4-4210) were obtained from Jeen International, Cerestar Company, and Dow Corning, respectively. They were used as supplied.

Preparation of nonoxynol-9 (N-9)-α-CD-IC

N-9-α-CD-IC was prepared by slowly adding 0.6 g of N-9 to 13.8 mL of an aqueous solution saturated with α-CD (4.0 g of α-CD) held at 60°C. After 2 h of stirring at 60°C, the covered flask was removed from the hot plate and left undisturbed overnight. A white precipitate was collected by filtration, and the crystals were washed with distilled water and dried.

Characterization

Thermal property analysis

DSC experiments were carried out on 3–10 mg samples with a Perkin–Elmer DSC 7 under nitrogen purge gas. A heating rate of 20°C/min was employed. Thermogravimetric analyzer (TGA) scans were obtained with a Perkin–Elmer Pyris 1 thermogravimetric analyzer on 5–10 mg samples. Samples were placed in an open platinum pan that was hung in the furnace. The weight percentage of remaining material in the pan was recorded during heating from 25 to 600°C at a heating rate of 20°C/min. Nitrogen was used as the purge gas.

X-ray diffraction

X-ray diffraction data were collected from powdered samples under the following conditions: Siemens type-F X-ray diffractometer, Ni-filtered Cu K_α with a wavelength of 1.54 Å, the voltage at 30 kV, the current at 20 mA, and scanning speed at $2\theta = 5°/\text{min}$ over the range $2\theta = 5–30°$.

Fourier transform infrared spectroscopy

Infrared spectra between 500 and 4000 cm^{-1} with a resolution of 2 cm^{-1} were recorded on a Nicolet 510P FTIR spectrometer. The α-CD and N-9-α-CD-IC samples were mixed with KBr and pressed into transparent disks, while a AgCl window was employed for the liquid N-9 sample.

Nuclear magnetic resonance spectroscopy

^{1}H-NMR spectra of N-9-α-CD-IC, α-CD, and N-9 were recorded on a Bruker Avance 500 MHz spectrometer in D_2O. One-dimensional ^{1}H data sets contained 16 K data points and sufficient scans were collected to obtain good S/N.

Preparation of silicone rubber films with/without pure N-9 or N-9-α-CD-IC

The silicone based elastomer and curing agent were thoroughly mixed in a 10 : 1 ratio with and without N-9 or N-9-α-CD-IC, as shown in Table I. During mixing, care was taken to minimize entrapment of air. The mixture was exposed to a vacuum of about 760 mmHg for approximately 10 min. Then, the vacuum was released for 1 min. This process was repeated three times and the sample cured for over 24 h at room temperature.

Analysis of bovine sperm motility and viability

Thawed frozen bovine semen from a single sire was used for analysis of all samples. For each replicate, five 0.5 mL semen straws were thawed for 30 s at 39°C, pooled and subjected to a swim-up procedure[19] to obtain motile spermatozoa. Swim-up selected motile bovine spermatozoa were washed and used at a final concentration of $3.88 \pm 0.53 \times 10^6$ sperm cells per milliliter. Circular inserts of silicone elastomer samples containing various concentrations of N9 were placed into individual wells of a 24-

TABLE I
Composition of Silicone Elastomers

Sample type	Silicone base (g)	Curing agent (g)	N9-α-CD-IC (g)	N9 (g)	wt % N9
SR-5% N9	10.0	1.0	0	0.0405	0.37
SR-10% N9	10.0	1.0	0	0.0810	0.73
SR-5% N9IC	10.0	1.0	0.5	0	~ 0.43
SR-10% N9IC	10.0	1.0	1.0	0	~ 0.83
SR-5% N9NMR	10.0	1.0	0	0.5	4.4

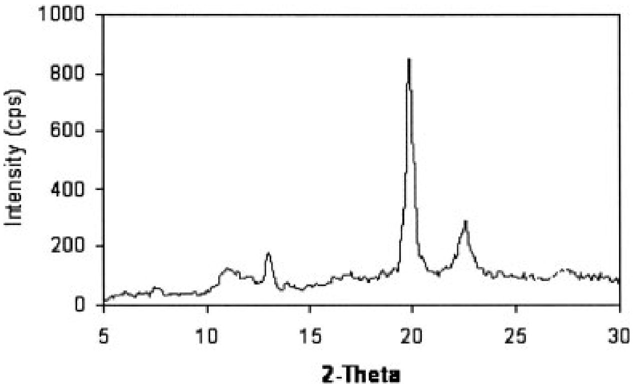

Figure 4 Wide angle X-ray diffraction of N-9-α-CD-IC.

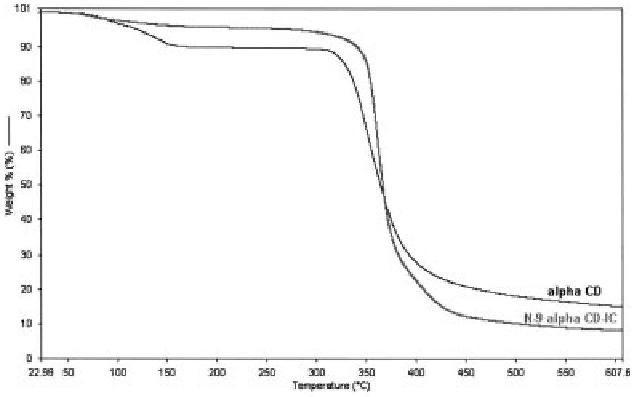

Figure 6 TGA scans of α-CD and N-9-α-CD-IC.

well culture plate and 100 μL of semen sample was added to each well on top of the silicone elastomer inserts. Semen samples were then collected at time 0 and at 10, 30 and 60 min after initial exposure to the elastomer inserts for analysis of sperm motility and viability. For all experimental replications ($n = 3$), the percent of motile sperm was determined by the same individual who had extensive experience evaluating livestock semen samples. The percent of viable sperm was determined based on counts of at least 100 cells from samples stained with eosin-nigrosin (live-dead) stain.

Percentage data for sperm motility and viability were analyzed by one-way analysis of variance using general linear models procedures from SAS.[20] When a significant F-statistic was encountered, means were separated by Duncan's multiple range tests. All data are presented as least squares means ± sem and a value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Characterization

N-9-α-CD-IC was characterized using FTIR, NMR, DSC, TGA, and wide angle X-ray diffraction to confirm IC formation. The X-ray diffractograms of the IC powders observed at wide angles were used for confirming the crystal structure of IC materials as shown in Figure 4. As is well-known,[21] the peak at $2\theta = 20^\circ$ in the wide angle X-ray diffraction of α-CD-ICs is characteristic for the channel structure of α-CD when including guest molecules. Therefore, the strong peak for N-9-α-CD-IC seen at approximately 20° (2θ) in Figure 4 indicates that the N-9-α-CD-ICs was formed in the channel crystalline structure.

The DSC technique was employed to determine whether the inclusion compound obtained contained free, uncomplexed N-9 guest. From Figure 5, we can see that pure N-9 melts at 5°C. The absence of the N-9 melting peak in the N-9-α-CD-IC scan indicates that there is no free N-9 in the N-9-α-CD-IC sample.

TGA was used to measure the thermal stability and decomposition behavior of N-9-α-CD-IC and α-CD, as shown in Figure 6. The N-9-α-CD-IC shows a higher decomposition temperature at 360°C compared with pure α-CD at ~ 350°C, consistent with the inclusion of N-9.

The FTIR spectra of α-CD, N-9, and N-9-α-CD-IC are presented in Figure 7. When the infrared spectra of α-CD and N-9-α-CD-IC are compared, they

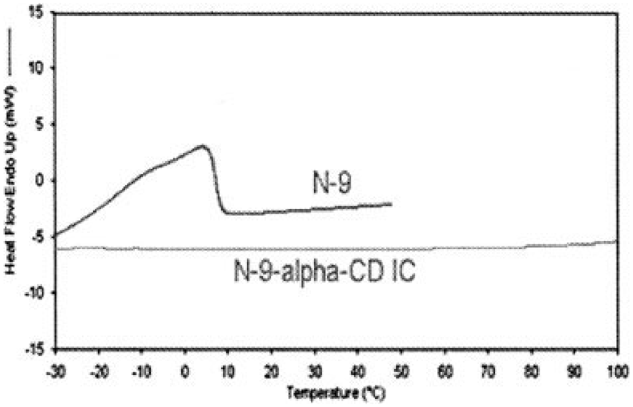

Figure 5 DSC scans of N-9 and N-9-α-CD-IC.

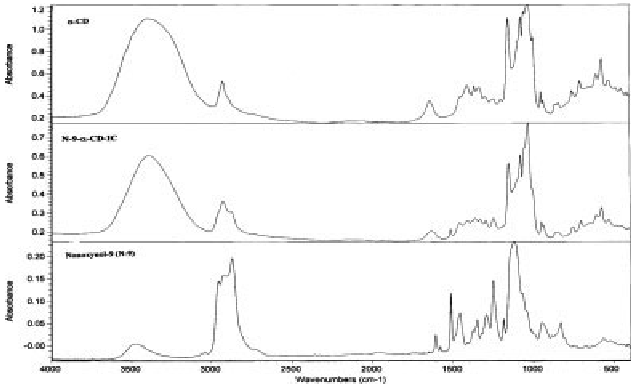

Figure 7 FTIR spectra of α-CD, N-9-α-CD-IC, and N-9.

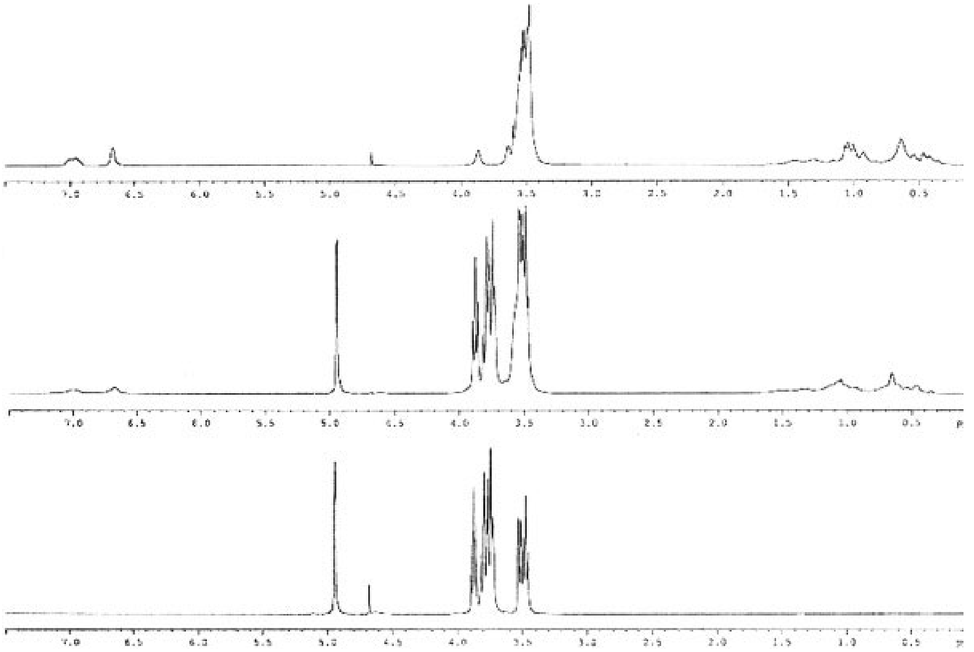

Figure 8 ^{1}H-NMR spectrum of N-9 (top), N-9-α-CD-IC (middle) and α-CD (bottom).

appear similar, although careful inspection of these solution spectra reveal subtle differences. The α-CD spectrum shows bands at 3390 and 2926 cm^{-1} because of the symmetric and antisymmetric O—H stretching modes and C—H stretching mode, respectively. Also, several peaks in the 1500–1200 cm^{-1} region are assigned to C—H, CH$_2$ and O—H bending modes. New bands appearing in the 2871 and 1512 cm^{-1} regions of the N-9-α-CD-IC are recognized as characteristic of N-9 and imply that N-9 is included inside the inclusion compound.

Furthermore, the N-9-α-CD-IC sample does not contain any free N-9 according to DSC. Hence, the observation of these new bands confirms that N-9 is included inside the channels provided by the IC. Therefore, we expect that N-9 is included inside the channels, which are provided by orderly stacked α-CD molecules and form the channel-type crystalline IC sample.

Further confirmation of the formation of an IC was observed in the ^{1}H-NMR spectra of α-CD, N-9-α-CD-IC, and N-9, as shown in Figure 8. New proton resonances in the N-9-α-CD-IC spectrum at about 7 and 6.5 ppm are contributed by the phenyl ring and —OH group of the N-9, respectively. In addition, several peaks in the 1.5–0.3 ppm region are contributed by CH$_2$ and CH$_3$ groups and also confirm the presence of N-9 in the N-9-α-CD-IC sample. ^{1}H-NMR resonance frequencies (chemical shifts) of pure α-CD are observed to be slightly shifted when

TABLE II
Effect of Various Silicone Rubber Films Swollen or Embedded with Pure N-9 or N-9-α-CD-IC on Motility and Viability of Bull Sperm at Different Times of Exposure

	Treatment*							Pooled SEM
	SR	SR-5%N9	SR-10%N9	SR-5%N9IC	SR-10%N9IC	SR-5%N9NMR	No Trt	
Percent motile cells								
0 min	$41.7^{a,**}$	38.3^{a}	26.7^{a}	26.7^{a}	28.3^{a}	0.0^{b}	46.7^{a}	8.2
10 min	40.0^{a}	10.7^{b}	$2.3^{b,c}$	$1.67^{b,c}$	$4.0^{b,c}$	0.0^{c}	40.0^{a}	5.8
30 min	21.7^{b}	0.3^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	41.7^{b}	4.2
60 min	8.7^{b}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	31.7^{a}	3.6
Percent live cells								
0 min	22.3^{a}	26.7^{a}	$11.0^{a,b}$	18.7^{a}	23.3^{a}	5.3^{b}	30.0^{a}	5.1
10 min	30.3^{a}	$14.0^{b,c}$	$17.3^{a,b}$	$14.0^{b,c}$	$14.3^{b,c}$	4.7^{c}	$27.0^{a,b}$	3.9
30 min	$20.0^{a,b}$	$14.7^{a,b}$	$6.7^{b,c}$	$5.3^{b,c}$	$7.0^{b,c}$	0.7^{c}	34.0^{a}	5.2
60 min	$8.3^{a,b,c}$	$3.0^{c,d}$	$5.3^{b,c,d}$	0.7^{d}	1.7^{d}	1.3^{d}	16.3^{a}	1.9

* Treatments: See Table I for treatment definitions.
** lsmeans.
$^{a,b,c,d}P < 0.05$, lsmeans with common subscripts do not differ between treatments within time of exposure.

N-9-α-CD-IC is dissolved, suggesting that even in solution N-9 is partially threading and complexing with α-CD.

Silicone rubbers swollen or embedded with pure N-9 or N-9-α-CD-IC

The effect of various silicone elastomers swollen or embedded with N-9 or N-9-α-CD-IC on bovine sperm motility and viability are summarized in Table II. Though not included in this table, silicone elastomers embedded with 5 and 10 wt % pure α-CD showed some spermicidal activity. Unlike the elastomers swollen or embedded with pure N-9 or N-9-α-CD-IC, however, after 30 min exposure ∼20% of bull sperm cells exposed to 5 or 10% pure α-CD remained motile (data not shown).

Presumably one of the factors influencing the efficacy of the N-9 spermicide in reducing the motility and killing sperm is the delivery system. The properties of the delivery system can influence the rate of diffusion of N-9 and contact with the sperm cells and possibly the biological action of the spermicide. Silicone rubber provides an elastomeric matrix that can either be swollen with pure N-9 or embedded with N-9-α-CD-IC crystals. The high mobility of the crosslinked chains in the silicone elastomer enabled the diffusion of N-9 spermicide to the outer surface of the elastomer, where it can contact the sperm plasma membranes. This resulted in a reduction of motility and reduced viability (death) of the sperm cells. Although the N-9 spermicide in the N-9-α-CD-IC are included in the host crystalline α-CD channels, they were apparently able to rapidly find their way to the surface of the silicone elastomer, because for the same N-9 content, silicone elastomers swollen with pure N-9 or embedded with N-9-α-CD-IC crystals showed virtually the same spermicidal activity after 10 min of exposure to sperm cells. Thus delivery of the N-9 spermicidal in the form of high melting N-9-α-CD-IC crystals provides a more convenient and possibly more permanent delivery that is equally effective as the pure liquid N-9 spermicidal. (Aging studies of the effectiveness of silicone elastomer films either swollen with pure liquid N-9 or embedded with N-9-α-CD-IC crystals are currently under way.)

CONCLUSIONS

We report the successful formation of the N-9-α-CD-IC. For the first time, we have observed the controlled release of N-9 spermicide from its α-CD-IC when embedded into silicone elastomers. The silicone elastomer embeddedd with N-9-α-CD-IC (0.44 wt % N-9) kills bovine sperm cells as well as the silicone elastomer swollen with pure liquid N-9 (0.37 wt % N-9). The results indicate that the silicone rubber with N-9-α-CD-IC was successful in destroying bovine sperm cells. The silicone elastomer's mobility and permeability enables both the pure liquid N-9 spermicide in the swollen elastomer and N-9 in its IC (embedded elastomer) to diffuse to the elastomer surface, where it contacts and kills the sperm. Our study may help in the design of future delivery systems for N-9 spermicides and to achieve improved condoms, because, unlike the pure liquid N-9, N-9-α-CD-IC crystals are high melting (above 250°C, see Fig. 6) and may be readily processed/embedded into polymer films and fibers that soften below this temperature. In the particular case of silicon rubber embedded with N-9-α-CD-IC, it remains to determine whether or not its mechanical/physical properties, such as elasticity, strength, and impermeability to sperm, will result in condoms and diaphragms that are not only spermicidal, but practical as well.

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