1-10-1995

High Energy Coprecipitate of Nonoxynol Oligomer, PVP and Iodine Having Contraceptive and Potent Anti-HIV Properties

George Digenis  
University of Kentucky, digenis@uky.edu

Alexander G. Digenis

Follow this and additional works at: https://uknowledge.uky.edu/ps_patents

Part of the Pharmacy and Pharmaceutical Sciences Commons

Recommended Citation  
https://uknowledge.uky.edu/ps_patents/87

This Patent is brought to you for free and open access by the Pharmaceutical Sciences at UKnowledge. It has been accepted for inclusion in Pharmaceutical Sciences Faculty Patents by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
United States Patent [19]
Digenis et al.

[54] HIGH ENERGY COPRECIPITATE OF NONOXYNL Oligomer, PVP and IODINE HAVING CONTRACEPTIVE AND POTENT ANTI-HIV PROPERTIES


[73] Assignee: University of Kentucky Research Foundation, Lexington, Ky.

[21] Appl. No.: 106,948

[22] Filed: Aug. 17, 1993

[51] Int. Cl. .......... A61K 47/32; A61K 31/085

[52] U.S. Cl. ............ 424/78.25; 424/DIG. 14;

[58] Field of Search .......... 424/78.24, 78.25, DIG. 14;

[56] References Cited

U.S. PATENT DOCUMENTS

4,297,341 10/1981 Waller et al.
4,775,638 10/1988 Haizma.
4,923,677 5/1990 Simon et al. .......... 422/37
4,925,033 5/1990 Stoner.
4,954,351 9/1990 Sackler et al.
5,070,889 12/1991 Leveen et al.
5,073,635 12/1991 Katz et al.
5,156,164 10/1992 Le Veen et al.

OTHER PUBLICATIONS


(Continued on next page.)

Primary Examiner—Thurman K. Page
Assistant Examiner—Peter F. Kulkosky
Attorney, Agent or Firm—Lowe, Price, LeBlanc & Becker

[57] ABSTRACT

A composition which is a contraceptive with potent anti-HIV activity. The composition is a high energy coprecipitate of nonoxynol-9 oligomers, polyvinylpyrrolidone (PVP) and iodine or PVP-I and shows a pronounced synergistic, anti-HIV effect between the compounds of the composition. A method of obtaining a contraceptive and anti-HIV environment in a female comprising administering to said female an effective amount of a high energy coprecipitate.

18 Claims, No Drawings
OTHER PUBLICATIONS


HIGH ENERGY COPRECIPITATE OF NONOXYNL OLIGOMER, PVP AND IODINE HAVING CONTRACEPTIVE AND POTENT ANTI-HIV PROPERTIES

TECHNICAL FIELD

The present invention relates to a composition which is a contraceptive with potent anti-HIV activity. The composition is a high energy coprecipitate of nonoxynol oligomers, polyvinylpyrrolidone (PVP) and iodine and shows a pronounced synergistic effect between the compounds of the composition.

BACKGROUND


By the nature of its synthesis, the nonoxynol-9 (N-9) (Igepal CO-630) derivative of nonoxynol is a polymer consisting of at least 17 oligomers of varying ethylene oxide (EO) chain length. The molecule of N-9 contains a hydrophobic moiety (nonylphenol portion) and a hydrophilic chain composed basically of ethylene oxide units. The compound is a product of a statistical polymerization reaction of 9 moles of ethylene oxide and one mole of nonylphenol (see equation below):

\[
\text{C}_9\text{H}_{19} \text{O} + \text{H}_2\text{C}+\text{O} \rightarrow \text{C}_9\text{H}_{19} \text{O}-(\text{CH}_2\text{CH}_2\text{O})_n-\text{H}
\]

where "n" represents the number of ethylene oxide units.

The above reaction does not yield a distinct compound but a mixture of oligomers with different molecular weights. The physical and chemical characteristics of these oligomers change as a function of the varying molecular weight. (See Liebert, M. A., (1983) J. Am. Coll. Toxicol. 2: 35-60). As the length of the EO chain increases, the oligomers increase in water solubility. Nonoxynol-9 oligomers 1 through 6 (n=1-6) are considered oil soluble compounds, whereas the oligomers with a longer EO chain are soluble in water and polar organic solvents (see Liebert, supra).

These differences in chemical properties of N-9 oligomers affect their biological behavior both in vitro and in vivo. For instance, it was noted that the dermal toxicity of nonoxynol decreases as the molecular weight increases (see Liebert, supra) and that smaller molecular weight nonoxynol may be more toxic to fibroblasts than the larger ones (see Chvapil, M. et al., (1980) Fertil. Steril. 33: 521-525).

The in vitro spermicidal activity of the N-9 surfactant is also related to its molecular weight. Thus the oligomer n=9 when separated from the N-9 compound, was much more effective in inhibiting the motility of the spermatozoon than the higher molecular weight nonoxynols where n=30, 50, and 100. (See Chvapil, M. et al., (1980) Fertil. Steril. 33: 521-525). The lower molecular weight nonoxynols (n=1 or 4) could not be studied appropriately because of their poor solubility in the aqueous testing medium (see Chvapil, supra).

Analogous dependence on molecular weight was observed in vivo. Oral absorption studies in the rat indicated that increasing the length of the ethylene oxide chain decreased N-9 oligomer intestinal absorption (see Knaak, J. B. et al., (1966) Toxicol. Appl. Pharmacol. 9: 331-340). Furthermore, data showed that absorption of N-9 through the vaginal membrane was poor and reflects the preferential absorption of hydrophilic low molecular weight oligomers. (See Walter, B. A. et al. (1988) Toxicol. Applied Pharmacol. 96: 258-268).


Utilizing this normal phase gradient elution HPLC method, at least seventeen oligomers were isolated from commercial N-9. Selected oligomers representing the high, medium and low molecular weight fraction of N-9 were separated in milligram quantities by normal phase gradient HPLC (see Walter, B. A. et al., (1991) Pharm. Res. 8: 409-411; and Walter, B. A. et al., (1991) Pharm. Res. 8: 403-408).

Polyvinylpyrrolidone (also known as povidone USP) is one of the most highly utilized polymers in medicine because of its safety for human use and unique hydrophilic properties (see Robinson, B. V. et al. (1990), A critical review of the Kinetics and Toxicology of Polyvinylpyrrolidone, Lewis Publishers, Inc., Michigan).

Discovered and used during World War II as a plasma expander, PVP is currently used as excipient in many pharmaceutical preparations intended for external use (e.g. povidone-iodine USP solutions such as Betadine); for oral use, such as a solubilizing agent and tablet disintegrant, and for vaginal use such as in PVP-I douche.


These studies found that the preferential dissolution of one component (hydrophilic polymer, such as PVP) can enhance the dissolution of the other component in a coprecipitate.

Drug/PVP high energy coprecipitates can be described as a drug incorporated into a solid PVP solu-
tion. Drug release from PVP coprecipitates are shown to follow dissolution kinetics of the polymer carrier of PVP provided that the PVP solvent uptake or swelling proceeds freely without inhibition by the drug.


The in vitro spermicidal activity of three molecular weight fractions of N-9 were compared to that of N-9, using rabbit spermatozoa, at equimolar concentrations. nonoxynol-9/PVP complexes were found to be far more effective in immobilizing the sperm than either N-9 alone or in the separate fractions (Walter, B. A. et al., (1991) Pharm. Res. 8: 403–408).

The spermicidal activities of three oligomeric fractions of N-9 with human sperm have been assessed. Equimolar concentrations of three different molecular weight fractions of N-9 coprecipitated with PVP were used. These equimolar concentrations were 166 µg/ml for high molecular weight (HMW), (MW=599), 123 µg/ml for middle molecular weight (MMW>MW=499) and 85 µg/ml for low molecular weight (LMW) (MW=306) N-9 fractions. The order of efficacy in immobilizing the human spermatozoa was HMW(MW=599)>MMW(MW=499)>LMW(MW=306) with complete sperm immobilization observed with PVP coprecipitated with N-9 HMW within 4.0 minutes and PVP coprecipitated with N-9 MMW within 15 minutes after exposure. Furthermore, addition of the spermicides interfered with the progressive motility and linearity of the sperm swimming pattern. PVP and buffer controls showed no decline in percentage motility over the course of the test.

Chvapil et al. recognized that the n=9 oligomeric fraction of nonoxynol-9 was more effective in inhibiting the motility of spermatozoa than the higher molecular weight nonoxynols. Chvapil et al., however, were unable to study the lower molecular weight oligomers (n=1–4) because of their poor solubility in aqueous media.

In contrast, however, Walter et al. were able to solubilize the water insoluble lower molecular weight N-9 oligomers by complexing them with the hydrophilic polymer polyvinylpyrrolidone (PVP). (See Walter, B. A. et al., (1991) Pharm. Res. 8: 403–408.)

The resulting high energy coprecipitate complexes of the low molecular species of N-9 were found to be at least as effective spermicides as all concentrations tested when compared to their counterparts that were prepared from higher molecular weight N-9 oligomeric fractions. However, they were themselves effective spermicides. The above findings concluded that when N-9 is coprecipitated with PVP its spermicidal activity is enhanced. While PVP alone has no inherent sperm toxicity, the formation of N-9/PVP complexes seem to produce a synergistic response which causes a more rapid damage to the sperm than any of the two materials alone (Walter, B. A. et al., (1991) Pharm. Res. 8: 403–408).


Unfortunately, N-9 causes epithelial disruption of the cervix and vagina when administered in high doses and high frequency (see Niruthisard S. et al., (1991) Sex. Transm. Dis. 18: 176–179). Higher rates of new HIV infections were found in prostitutes who used N-9 at great frequencies (see Kreiss, J. et al., International Conference on AIDS, Montreal, June 1989 [Abstract MAO36]). This observation was attributed to the high incidence of genital ulceration caused by high doses of N-9, in this group of women. Thus, the above findings tend to suggest that novel spermicide formulations containing N-9 should be efficacious at the smallest possible doses so that the integrity of the vaginal epithelium is not compromised.

The antimicrobica properties of povidone-iodine (PVP-I), a complex of polyvinyl pyrrolidone and iodine, have been well documented. PVP-I solutions (10% w/v) USP are among the most widely utilized antimicrobial agents. A 10% (w/v) solution of PVP-I contains 1% (w/v) of available iodine (I2). The microbiological potency of PVP-I arises from the elemental (diatomic) or free iodine (I2) in solution. The significant characteristic of iodophores, such as PVP-I, is that they carry almost all of their iodine in a complexed form so that the amount of free iodine (I2) is quite low and PVP-I serves as the iodine reservoir delivering the free iodine into the solutions. Thus, iodophors exhibit reduced irritation properties and are relatively non-toxic (see LaRocca, R. et al., (1983) in Proceedings of the International Symposium on Povidone, Degenis, G. A. and Ansell, J., Eds. Lexington, pp. 101–119).

Stable, sterile (0.2%) PVP-I compositions containing little as 0.02% iodine have been shown to be useful in treating eye infections in humans. A level of 0.02% iodine obtained by diluting a commercial 10% PVP-I solution at 1:50 with saline solution, is generally considered to be optimum to maximize performance and minimize irritation (see Winicov, M. et al., (1987) in Proceedings of the International Symposium on Povidone, Degenis, G. A. and Ansell, J., Eds. Lexington, pp. 57–64).

Data has shown that with doubly labeled 14C-PVP-131I solutions the amount of iodine delivered into gram positive and negative bacteria cultures was three times greater when the iodine was complexed with PVP, than from an equimolar solution of 131I—(Lugol's solution). (See Degenis, G. A. et al., (1983) in Proceedings of International Symposium on Povidone, Degenis, G. A. and Ansell, J., Eds. Lexington, pp. 302–311).

The hydrophilic polymer PVP acts as a delivery system for iodine probably due to the membrane seek-
5,380,523

5

ing properties of this polymer. Ben-David and Gavendo have shown that PVP at 4.6% w/v concentrations protect red blood cells from osmotic fragility and mechanical injury. (See Ben-David A. et al., (1972) *Cryobiology* 9: 192–197). These workers suggested that this effect is brought about by a "coating" or external interaction of PVP with cell membranes.

The membrane-seeking properties of PVP suggest that in addition to its contribution to the solubilization ability of N-9, the PVP polymer, via its cell-membrane coating properties, also provides vaginal and cervical surface coverage coating with N-9 and iodine over extended periods of time.

In addition to its antimicrobial properties, PVP-I has been shown to inactivate HIV. (See Kaplan, J. C. et al. (1987) *Infect. Control* 8: 412–424; and Harbison, M. A. et al., (1989) *J. Acquir. Immune Defic. Syndr.* 2: 16–20.) The concentration of iodine used in Kaplan's studies was equal to 0.052% for 250 ppm of I2, and

A 0.02% w/v (200 ppm) solution of iodine is considered non-toxic and non-irritating and is used for treatment of eye infections in humans. (See Wincov, M. et al. (1987) in *Proceedings of the International Symposium on Povidone* Digenis, G. A. and Ansell, J., Eds. *Lexington* pp. 57–64.) In fact, the increased bactericidal activity of dilute solutions of povidone-iodine (Betadine—10% w/v PVP-I) have recently been well documented. Betadine contains 10,000 ppm (or 10,000 micrograms/ml) of available iodine and is often irritating to the tissues and has an undesirable brown color. (See Berkman, R. L. et al., (1982) *J. Clin. Microbiol.* 15: 635–639.) At concentrations of about 0.02% w/v of iodine, the undesirable brown color of iodine is not a problem since in dilute solutions the color is hardly seen and the amount of iodine is not irritating to tissues.

None of the prior research in this area recognized the synergistic anti-HIV result of all three compounds when formulated into a high energy coprecipitate.

Furthermore, spermicides containing nonoxynol-9 and polyvinylpyrrolidone or polyurethane are known. U.S. Pat. No. 4,317,447 to Williams discloses a device for delivering a medicament to the vaginal cavity consisting of a molded sheath of a mixture of a polymeric material and a medicament. The medicament which is dispersed in the polymeric material can be nonoxynol-9. The polymer may be selected from modified cellulose, polyvinyl alcohol, polyvinylpyrrolidone, polyacrylic and ethylene oxide polymer. Williams does not disclose the use of PVP-I in combination with a nonoxynol-9.

U.S. Pat. No. 5,156,164 to LeVeen et al. discloses that iodine can be dissolved in alcohol containing nonoxynol in a complex with polyurethane. LeVeen et al. disclose that a polyvinylpyrrolidone-iodine complex has been effective in treating resistant vaginitis. LeVeen et al. do not disclose or suggest combining PVP-I with nonoxynol.

U.S. Pat. No. 5,070,889 to Leven discloses a contraceptive sponge and tampon made of polyurethane iodine complex. Leven et al. teaches away from the use of povidone iodine or use as a contraceptive.

U.S. Pat. No. 5,073,365 to Katz et al. discloses clinical and personal care articles enhanced by lubricants and adjuvants. The devices can be made of polyvinylpyrrolidone or polyurethane interpolymer. The device may take the form of vaginal diaphragms, tampons, condoms or cervical caps. The medicament may be nonoxynol-9. Katz et al. disclose that the personal care articles may prevent the transmission of venereal diseases, possibly including AIDS.

U.S. Pat. No. 4,707,362 to Nuwayser discloses a sustained release composition made of synthetic polymers such as polyvinylpyrrolidone. The bioerodible material in one embodiment has been modified so that a spermicide such as nonoxynol-9 is slowly released. Nuwayser does not disclose PVP-I in combination with nonoxynol-9.

U.S. Pat. No. 4,954,351 to Sackler et al. discloses a method of producing standardized povidone-iodine preparations. The patent discloses that the povidone-iodine solution can be incorporated into a suppository with 0.1 to 10% by weight of povidone-iodine. Sackler et al. do not disclose the use of povidone-iodine in combination with nonoxynol-9.

U.S. Pat. No. 4,297,341 to Waller et al. discloses that a water-soluble complex comprising polyvinylpyrrolidone and gossypol is an effective spermicide. The patent tentatively discloses that a PVP-gossypol combination when compared to a comparative example of nonoxynol-9 alone, exhibited equal or greater spermicidal properties. Waller does not disclose PVP-I in combination with nonoxynol-9.

U.S. Pat. No. 4,925,033 to Stoner et al. discloses a microbicidal cleanser/barrier kit. One method of the invention involves applying a solution of povidone-iodine (PVP-I) to vaginal sponges or condoms. In another embodiment the povidone-iodine active ingredient may be added to spermicidal birth control compounds. Stoner et al. disclose that nonoxynol-9 is a known spermicidal compound. Stoner et al. do not disclose or suggest the particular combination of components in the form of a high energy coprecipitate, nor that the compounds show a synergistic anti-HIV effect.

Heterosexual transmission of human immunodeficiency virus (HIV), the causative agent of AIDS, is a growing concern in the United States where 37% of AIDS cases are heterosexually transmitted, the majority being male-to-female. Moreover, the frequency of global heterosexual transmission is probably greater where it is estimated to exceed 60% of all AIDS cases. It has been suggested that a major contributing factor to heterosexual transmission of HIV is the presence of cell-free virus and virus-infected cell (cell-associated virus in genital secretions. Thus, vaginal contraceptives which inactivate HIV should be an effective barrier to transmission.

There is a need in the pharmaceutical area for a contraceptive with potent anti-HIV activity. Ideally, a contraceptive agent is needed that can:

1a) provide rapid spermicide delivery (within 30–60 seconds);

1b) provide long-acting spermicidal activity after a single application;

2) protect the user against HIV (AIDS) and other sexually transmitted diseases (STDs);

3) protect vaginal and cervical epithelia from irritation;

4) enhance penetration of spermicide into cervical mucus;

5) exhibit low systemic bioavailability (low absorption);

6) be pharmaceutically and cosmetically acceptable (e.g. a tablet or capsule are preferred, as suppositories, creams and gels appear not to be as attractive to young users).
The composition of the invention meets the above objectives and provides a high energy coprecipitate of nonoxynol-9 oligomers, polivinlypyrrolidone and iodine (PVP-I). The composition shows a pronounced synergistic effect between the compounds which results in potent anti-HIV activity. The composition of the invention safely and cost-effectively provides a contraceptive with potent anti-HIV activity.

Disclosure of the Invention

It is an object of the invention to provide a novel and potent contraceptive and/or spermicide possessing pronounced anti-HIV properties which is a high energy coprecipitate composition comprising N-9 oligomers and PVP and iodine. The PVP and iodine ingredients may be present in the coprecipitate composition as the complex PVP-I.

The invention further provides a high energy coprecipitate composition which is in the form of a powder. In a preferred embodiment the invention provides a high energy coprecipitate composition including PVP-I which comprises about 0.09–9% w/w of iodine.

In yet another embodiment, the invention provides a high energy coprecipitate composition of N-9 complexed with PVP comprising an antiviral concentration of free iodine of about 0.03% w/v or about 300 ppm.

The invention additionally provides a high energy coprecipitate composition of N-9 complexed with PVP comprising an antiviral concentration of free iodine providing about 9.4 to 0.09 % I;) providing an effective concentration of free iodine of 0.0094% w/v (94 ppm) and from about 11.8–5.8% w/w of N-9 providing an effective concentration of N-9 of 0.0058% w/v (58 ppm).

In a further embodiment, the invention includes a high energy coprecipitate composition comprising an effective final concentration of from about 4–200 μg/ml of N-9.

The invention advantageously provides for a method of preparing a high-energy coprecipitate composition comprising the steps of:

(a) fractionating commercially available spermicide N-9 to seventeen oligomers by a preparative high pressure liquid chromatography (HPLC) procedure; and

(b) converting the oligomers or commercially available N-9 to a high energy coprecipitate with PVP and iodine.

Preferred is a method of preparing a high-energy coprecipitate composition including the steps of:

(a) fractionating commercially available spermicide nonoxynol-9 (N-9) to seventeen oligomers by high pressure liquid chromatography (HPLC);

(b) adding an N-9 oligomer obtained in step (a) with a 10% or 1% solution w/v of PVP-I in a solvent,

(c) placing the product of step (b) in an oil bath at about 100°C, and allowing the solvent to evaporate.

(d) obtaining a high energy coprecipitate of N-9, polivinlypyrrolidone (PVP) and iodine.

Also included is a method of treating a female comprising administering to a female an effective amount of a high energy coprecipitate comprising N-9 oligomers, PVP and iodine to achieve a contraceptive and anti-HIV environment in the vaginal cavity. Thus, the invention provides spermicidal and anti-HIV activity with no irritation to the cervical and vaginal epithelia, and may take the form of an elegant, free flowing powder.

DESCRIPTION OF THE INVENTION

The present invention describes methodology for the preparation and assessment of new chemical entities with pronounced spermicidal and anti-HIV properties. These chemical entities incorporate N-9 or selected N-9 oligomers, small amounts of iodine (I) and the hydrophilic polymer PVP in such a way as to form high energy complexes, also known as coprecipitates.

The novel contraceptive composition of the invention comprises a high energy coprecipitate made from N-9 oligomers, PVP and iodine. A pronounced synergistic effect is obtained between the compounds, enhancing the anti-HIV activity of the compounds when in the form of a coprecipitate. Due to the synergistic effect of the compounds present in the high energy coprecipitate, the coprecipitate requires less of each of the individual ingredients to achieve the desired anti-HIV result, than if the compounds were to be administered as a mixture. This is beneficial as it reduces irritation to tissues caused by higher doses of N-9 and iodine and thus reduces the potential for HIV virus transmission.

The high energy coprecipitates of the invention are formulated by fractionating commercially available spermicide, N-9, into seventeen oligomers by a preparative high pressure liquid chromatography (HPLC) procedure. Selected oligomers of N-9, or the commercially available N-9, are converted to high energy coprecipitates with PVP and various amounts of iodine (PVP-I). In a preferred embodiment the amount of iodine is about 0.09–9% w/w of iodine. The content of N-9 in the above complexes, in a preferred embodiment, ranges from 5.8–12% w/w.

N-9 and its oligomeric components are inherently viscous liquids which are converted to free flowing, water soluble powders by forming coprecipitates with PVP-I. Several combinations of iodine (I), N-9 and PVP are utilized to produce powders which exhibit a variety of spermicidal and anti-HIV activities.

In order to test and compare the anti-HIV activity of the high energy coprecipitates of the invention, the following assay procedure was performed.

Procedure for Anti-HIV Activity Assessment

High-titer suspensions (> 10⁶ infectious particles/ml) were incubated with each compound for 5 seconds, then immediately diluted 1:50 onto cultures of human MT-2 cells for detection of infectivity. Cellular toxicities were observed and no antiviral activities were expected after the 1:50 dilution, hence all antiviral activity would have resulted from the 5 second exposure. Infections were monitored by syncytium (giant cells) formation and Immune Fluorescence Assay (IFA). This assay utilizes all antiviral antibody with a fluorochrome attached to it through a covalent bond. Virus particles get to fluoresce when viable, and therefore the degree of infectivity is quantitated.

The results after 3, 5 and 8 days of incubation are shown in Table 1. In the control experiment, using the untreated virus, the MT-2 cells are 100% infected at the end of 3 days.

A 1% value or less, indicates a complete inactivation of the virus as measured by the IFA method, i.e. no fluorescent particles of the virus exist inside or outside the cells (Table 1).
Syncytia (giant cell) formation is assessed by staining techniques with subsequent microscopic examination. Syncytia formation indicates invasion of the MT-2 cells by the virus.


Table 1 summarizes the results of anti-HIV activity assessment of two high energy coprecipitate powders of PVP-I and N-9. The first was made with oligomers of N-9 with an average molecular weight of 599 and the second with the oligomers of N-9 with an average molecular weight of 306. Both samples were coprecipitated with PVP, iodine and one of N-9 oligomers mentioned above.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>3 days</th>
<th>5 days</th>
<th>8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Syncytia</td>
<td>IFA</td>
<td>Syncytia</td>
</tr>
<tr>
<td>PVP (11.8 mg/ml)</td>
<td>++ +</td>
<td>80%&gt;</td>
<td>CPE</td>
</tr>
<tr>
<td>PVP (0.236 mg/ml)</td>
<td>++ +</td>
<td>&gt;80%</td>
<td>CPE</td>
</tr>
<tr>
<td>PVP (3.24 mg/ml)</td>
<td>10%</td>
<td>++ +</td>
<td>&gt;80%&gt;</td>
</tr>
<tr>
<td>high MW N-9 (0.2 mg/ml)</td>
<td>+ + +</td>
<td>&gt;80%</td>
<td>CPE</td>
</tr>
<tr>
<td>PVP (0.0648 mg/ml)</td>
<td>+ + +</td>
<td>&gt;80%</td>
<td>CPE</td>
</tr>
<tr>
<td>high MW N-9 (0.004 mg/ml)</td>
<td>10%</td>
<td>++ +</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>PVP (5.8 mg/ml)</td>
<td>low NW N-9</td>
<td>(0.2 mg/ml)</td>
<td>+ + +</td>
</tr>
<tr>
<td>PVP (0.116 mg/ml)</td>
<td>low NW N-9</td>
<td>(0.004 mg/ml)</td>
<td>+ + +</td>
</tr>
<tr>
<td>PVP-I (11.8 mg/ml)</td>
<td>+ + +</td>
<td>&gt;80%</td>
<td>CPE</td>
</tr>
<tr>
<td>PVP-I (0.236 mg/ml)</td>
<td>1-1%</td>
<td>++ +</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>PVP-I (3.24 mg/ml) high MW N-9</td>
<td>(—)</td>
<td>=&lt;1%</td>
<td>+ + +</td>
</tr>
<tr>
<td>PVP-I (0.2 mg/ml)</td>
<td>(0.0648 mg/ml)</td>
<td>high MW N-9</td>
<td>(0.004 mg/ml)</td>
</tr>
<tr>
<td>PVP-I (5.8 mg/ml) low NW N-9</td>
<td>(0.2 mg/ml)</td>
<td>+ + +</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>PVP-I (0.116 mg/ml) low NW N-9</td>
<td>(0.004 mg/ml)</td>
<td>+ + +</td>
<td>&gt;80%</td>
</tr>
</tbody>
</table>

The data in Table 1 show that PVP-I at a concentration of 11.8 mg/ml completely inactivated cell-free and cell-associated HIV-1. This represents approximately an effective concentration of 1.2% w/v of PVP-I or 0.12% w/v of free iodine (or 1,200 ppm of I2).

A solution was made from a PVP-I and N-9 high energy coprecipitate. The coprecipitate composition was 94.2% w/w of PVP-I and 5.8% w/w of N-9, a concentration of 3.24 mg/mL of PVP-I and 0.2 mg/mL of N-9 of an average molecular weight of 599. This composition achieved a complete eradication of the HIV virus in 30 seconds.

The use of high molecular weight N-9 oligomers as a component of the anti-HIV coprecipitate composition decreases the systemic absorption of N-9 and therefore N-9 is localized in the vagina and less toxic to the female.

Similar anti-HIV results were obtained with a coprecipitate of about 5.8 mg/mL PVP-I and about 0.2 mg/mL N-9 of an average molecular weight of 306. These results represent an antiviral concentration of free iodine of about 0.03% w/v (324 ppm). This level of iodine is not irritating to the vaginal epithelial tissue and desirably has no noticeable brown color.

In contrast to the above results, when no iodine is present in a coprecipitate composition made with 94.2% w/w of PVP and 5.8% w/w of N-9, at a concentration of 3.24 mg/mL of PVP and 0.2 mg/mL of N-9 of an average molecular weight of 599, the antiviral properties of the product were substantially reduced (see Table 1).

A similar reduction of the antiviral properties was also observed when the product was made from a PVP/N-9 high energy coprecipitate of 94.2% w/w of PVP and 5.8% w/w of N-9, at a concentration of 3.24 mg/mL of PVP and 0.2 mg/mL of N-9 of an average molecular weight of 599 (see Table 1).

Thus, in a preferred embodiment, the presence of 324 ppm iodine in the case of high molecular weight (599) N-9 and 580 ppm of iodine, in the case of the lower molecular weight N-9 (306) importantly adds to the synergistic anti-HIV effect of the high energy coprecipitate. The iodine and N-9 both act in a synergistic manner when complexed with PVP.

Other solutions made from PVP-I and N-9 coprecipitate in accordance with the invention include 94.2% of PVP-I and 5.8% of N-9 with an average molecular weight of 599. A further solution made from PVP-I and N-9 coprecipitate comprises 94.2% of PVP and 5.8% of N-9 with a weight average molecular weight of 306.

The chemical union of the iodine, PVP and several oligomers of the spermicidal agent N-9 with different molecular weights results in an impressive synergistic action of iodine and the spermicide N-9 against human immunodeficiency virus (HIV) (Tables 1 and 2).

The coprecipitate of the invention shows that the synergism between iodine and N-9 is of such magnitude that solutions with even smaller concentrations of these species can be used. Again, this is beneficial as it reduces vaginal irritation and thus reduces the potential for HIV virus transmission. Indeed, a complete eradication of the HIV-virus was obtained when coprecipitates of PVP, iodine and N-9 solution, containing as low as 58 ppm of N-9 and 940 ppm of PVP-I (94 ppm of iodine), were utilized (see Table 2).

TABLE 2

<table>
<thead>
<tr>
<th>Concentration on Virus</th>
<th>Syncytia</th>
<th>IFA Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>[µg/mL = ppm]</td>
<td>Syncytia</td>
<td>IFA Position</td>
</tr>
</tbody>
</table>

1 Details of experimental procedures are given in the text.
2% IFA positive cells
3% CPE, virus-induced cytopathic effect was complete.

HMW N-9 = 599
LMW N-9 = 306
TABLE 2-continued

<table>
<thead>
<tr>
<th>Compound</th>
<th>N9</th>
<th>PVP-I</th>
<th>% Cells</th>
<th>% No drug</th>
<th>—</th>
<th>—</th>
<th>100</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>KY048</td>
<td>58</td>
<td>940</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>KY050</td>
<td>117.4</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>KY052</td>
<td>118</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Composition of KY Coprecipitates

<table>
<thead>
<tr>
<th>PVP</th>
<th>PVP-I</th>
<th>I2</th>
<th>N-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>----</td>
<td>-------</td>
<td>----</td>
<td>-----</td>
</tr>
<tr>
<td>KY048</td>
<td>0</td>
<td>94.20</td>
<td>940,000</td>
</tr>
<tr>
<td>KY050</td>
<td>88.58</td>
<td>885,800</td>
<td>0.90</td>
</tr>
<tr>
<td>KY052</td>
<td>88.20</td>
<td>882,000</td>
<td>0</td>
</tr>
</tbody>
</table>

The synergistic effect against the HIV is so significant that the above PVP-I/N-9 solution at concentrations as small as 58 ppm of N-9 and 94 ppm of iodine are effective, against the HIV virus (Table 2).

In addition to the unexpected synergy between the compounds, the coprecipitation of iodine, N-9 and PVP results in the formation of freely flowing powders which enable the preparation of elegant solid pharmaceutical formulations with control release properties. The coprecipitate powders are particularly useful in the design of acceptable solid formulations intended for vaginal, or other appropriate use.

The coprecipitate composition may be administered in the form of solid formulations, such as tablets or capsules, or as ointments, creams or suppositories. In a preferred embodiment the composition is administered in the form of a capsule or tablet.

Tablets, capsules, ointments, creams or suppositories may be formulated according methods known in the art such as those disclosed in Remington's Pharmaceutical Science, 18th Ed. pp. 1602-1712, incorporated herein by reference.

In contrast, N-9 by itself is a viscous liquid which cannot be formulated as a powder in solid dosage forms such as tablets or capsules. N-9 has heretofore been unable to form elegant tablets or capsules which are preferred by the user cover creams and suppositories. Additionally, at its generally accepted effective spermicidal concentrations (approximately 160 mg/ml), N-9 is irritating to the vaginal epithelium and lining of the vaginal vault.

Concentrations below 550 ppm neither iodine nor N-9 are known to irritate sensitive membrane linings of the human body. Thus, the coprecipitate powders can be formulated into drug delivery systems that can release their active ingredients in a predictable manner without causing irritation to surrounding membrane linings.

The powders of the invention are acceptable to the user and furnish prolonged protection against AIDS and sexually transmitted diseases (STDs) with minimal absorption to the systemic circulation.

The main ingredient in the powders of the high energy coprecipitates, described in this invention, is the hydrophilic polymer PVP. This polymer, which exists in the powders in a ratio of approximately forty parts to one part of N-9, not only enhances the solubility of the various N-9 oligomers but also contributes to the synergistic action of N-9 and iodine against the AIDS virus. Thus, in a most important aspect of the invention, the active ingredients (N-9 and iodine) are present in the powder in such minuscule quantities that are known not to irritate membrane linings of the body.

The following examples are given to illustrate the invention, but are not to be construed as limiting.

EXAMPLE 1

The various compositions of coprecipitates are made according to the following general procedure: Stock solutions of approximately 1% w/v of PVP-I and 1% w/v N-9 are made in ethanol or methanol.

The N-9 stock solution is added dropwise to an aliquot of the PVP-I stock solution with stirring. It is critical that the two solutions are mixed at a very slow rate. Thus, the introduction of the N-9 solution, into the round bottom flask, containing the PVP-I, must be done dropwise but over a period of not longer than 1.5 hours.

Subsequently, the solvent is evaporated from the PVP-I/N-9 solution by heating in a preheated oil bath at 100° C. The temperature must be kept constant at 100° C. The evaporation process should last between 1-2 hours but no longer than two hours.

EXAMPLE 2

If a "glassy" coprecipitate is obtained following the general procedure described in example 1, diethyl ether is mixed with the residue. Upon evaporation of the diethyl ether a crystalline powder is obtained.

EXAMPLE 3

If a finely divided crystalline powder of the PVP-I/N-9 coprecipitate is desired, following the procedure described in Example 1, the residue, received after evaporation described in Example 1, is dissolved in a mixture of methanol/1,4 dioxane (1:4 ratio v/v) and subjected to freeze-drying for at least 12 hours. A fine crystalline powder is obtained by the freeze-drying step.

EXAMPLE 4


Briefly, the separation is achieved by a preparative Zorbax-NH2 column (250 mm, 7 μm, ×21.1 mm i.d.).

EXAMPLE 5

Stock solutions of N-9 (250 mg/ml) were prepared in tetrahydrofuran. A 1.0 ml aliquot was separated on a preparative Zorbax-NH2 7 μm column, 250 mm ×21.2 mm i.d., using a linear solvent gradient from 98% A-2% B to 50% A-50% B in 90 min., where A=tet-
5,380,523 13
rhydrofuran:hexane (20:80, v/v) and B=water:2-
propanol (10:90, v/v) delivered at 9.9 ml/min at ambi-
tent temperature and detection at 280 nm. The HPLC
system used was a Waters HPLC system (Millipore,
Waters Chromatography Division, Millford, Mass.)
consisting of two Waters Model 510 HPLC pumps, a
Waters Model 680 gradient controller, a Waters Model
440 Absorbance detector (280 nm), and a Waters Model
SE120 dual channel recorder. Samples were introduced
via a Rhodyne Model 7125 loop injector equipped
with a 100 μl loop onto the HPLC column. Seventeen
oligomers were collected, concentrated and reinfected
onto the analytical system for further purification. The
599 molecular weight fraction and 305 molecular
weight fraction were separated from the other fractions.
A 10% or 1% solution of w/v of PVP-I and the MW
599 fraction of the compound of nonoxynol-9 (N9)
(isolated as set forth above) in absolute ethanol (metha-
nol) was made. Both solutions were added via dropping
funnels into a round bottom flask. It is important in this
step to allow the dropping of the two solutions to con-
tinue for 1.5 hours. Thus the addition of the two solu-
tions must take place slowly.
To an oil bath which had been preheated to 100° C.,
the flask was placed and the solvent allowed to evapo-
rat. It was important to make sure that the tempera-
ture was well controlled during the evaporation process.
The evaporation process should take between 1 and 2
hours. The evaporation should not take place longer
than 2 hours.
A high energy coprecipitate of the compounds is
obtained.
Examples 6–8 show formulations of the high energy
coprecipitate of the invention.

EXAMPLE 6
A high energy coprecipitate composition of
9.4–0.09% I w/w and 11.8–5.8% N-9 w/w. The high
energy coprecipitate provides anti-HIV protection at an
effective concentration of about 3.24 mg/ml of PVP-I
and 0.2 mg/ml of N-9 of an average molecular weight
of 599 in a tablet formulation.

EXAMPLE 7
A high energy coprecipitate providing anti-HIV pro-
tection at an effective concentration of about 5.8 mg/ml
PVP-I and 0.2 mg/ml N-9 of an average molecular
weight of 305 in a capsule formulation.

EXAMPLE 8
A high energy coprecipitate providing anti-HIV pro-
tection at an effective concentration of about 58 ppm
of N-9 and 940 ppm of PVP-I or 94 ppm of iodine in a
cream formulation.
The invention is not to be limited to the exact details
of operation, or to the exact compositions, methods,
procedures or embodiments shown and described, as
obvious modifications and equivalents will be apparent
to one skilled in the art. The invention is therefore to be
limited only by the full scope which can be legally
 accorded to the appended claims.
We claim:
1. A composition comprising a high energy copreci-
pite of nonoxynol oligomer and polyvinylpyrrolidone
polymer and iodine, or polyvinylpyrrolidone-iodine
complex (PVP-I), wherein the combined effect of the
compounds simultaneously precipitated in said high
energy coprecipitate exceeds the individual effect of
said compounds, and wherein said high energy copre-
cipitate has antiviral activity against human immu-
nodeficiency virus-1 (HIV-1) in an MT-2 assay.
2. A composition according to claim 1, wherein said
coprecipitate is a powder.
3. A composition according to claim 2, wherein said
powder is a finely divided crystalline powder.
4. A composition according to claim 1, wherein said
PVP-I comprises about 0.09–9.4% w/w of iodine.
5. A composition according to claim 1, wherein said
PVP-I comprises an antiviral concentration of free
iodine of about 0.03% w/v or 300 ppm complexed with
PVP.
6. A composition according to claim 1, wherein said
PVP-I comprises an effective antiviral concentration
of free iodine of about 0.0094 w/v of iodine or 94 ppm
complexed with PVP.
7. A composition according to claim 1, wherein said
nonoxynol oligomer of said coprecipitate comprises
from about 5.8 to 11.8% w/w of nonoxynol-9 oligomer.
8. A composition according to claim 1, wherein said
coprecipitate is present at a concentration of about
3.24 mg/ml of PVP-I and about 0.2 mg/ml N-9 oligomer
of an average molecular weight of 599.
9. A composition according to claim 1, wherein said
coprecipitate is present at a concentration of about
5.8 mg/ml PVP-I and about 0.2 mg/ml N-9 oligomer of
an average molecular weight of 305.
10. A composition according to claim 1 wherein said
coprecipitate is present at a concentration of about
5.8 ppm of N-9 oligomer and about 940 ppm of PVP-I or 94
ppm of iodine.
11. A composition of claim 1, wherein said nonoxynol
oligomer is an nonoxynol-9 oligomer with an average
molecular weight of 599.
12. A composition of claim 1, wherein said nonoxynol
oligomer is nonoxynol-9 oligomer comprising oligo-
ers of N-9 having a range of molecular weight of each
oligomer from about 264 to about 1000.
13. A composition of claim 1, wherein the percent of
polyvinylpyrrolidone ranges from about 88% to about
97%.
14. A composition of claim 1, wherein the average
molecular weight of said polyvinylpyrrolidone ranges
from about 2,500 to 1,100,000.
15. A method of preparing a composition according
to claim 1, comprising the steps of:
(a) fractionating commercially available spermicide
nonoxynol-9 (N-9) to seventeen oligomers by high
pressure liquid chromatography (HPLC);
(b) adding an N-9 oligomer obtained in step (a) with
a 10% or 1% solution w/v of PVP-I in a solvent,
(c) placing the product of step (b) in an oil bath at
about 100° C., and allowing the solvent to evapo-
rate; and
(d) obtaining a high energy coprecipitate of N-9,
polyvinylpyrrolidone (PVP) and iodine.
16. A method of achieving a contraceptive and anti-
HIV in environment the vaginal cavity of a female
comprising administering to said vaginal cavity of said
female an effective amount of a composition according
to claim 1 to achieve a contraceptive and anti-HIV in
environment the vaginal cavity of said female.
17. The method according to claim 16, wherein said
coprecipitate is administered in a form selected from
the group consisting of tablets, capsules, ointments, creams
and suppositories.
18. The method according to claim 16, wherein said
effective amount of said coprecipitate comprises from
about 6–12% w/v N-9 and about 0.09–9% w/v of PVP-
1.