SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES

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Abogado
MAURICIO JARAMILLO CAMPUZANO

ASUNTO

Radicación	08 078 520
Trámite	011
Evento	379
Actuación	519
Oficio	E13039
Folios	12

Patente de Invención	☑		Patente de Modelo de Utilidad
Vía Nacional			
Vía PCT (Fase Nacional)	☑	Capítulo I	Capítulo II ☑
No. Solicitud Internacional		PCT/GB2006/004775	
Fecha de Presentación Internacional		19/diciembre/2006	

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 28 a 41	29/julio/2008
Reivindicaciones:	Folios 42 a 48	29/julio/2008
Prioridad:	GB 0526508.3	29/diciembre/2005

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Fusión de solicitudes en trámite (Art 37 D 486)

Teniendo en cuenta que ante la Superintendencia se presentan las solicitudes 08-78513 y 08-78520 que comparten materia, debido a que como se demuestra en la tabla 1 no sólo comparten ingredientes sino el mismo concepto de formulación en polvo.

Tabla 1

08-78513	08-78520
Rv. 1 Polvo hemostático que comprende una sal de chitosán y al menos un surfactante médico.	Rv. 1 Polvo hemostático que comprende una sal de chitosán y al menos un material inerte.
Rv.2 Donde la sal de chitosan comprende uno o más del grupo que comprende uno o más del grupo consistente de en: acetato de chitosan, lactato de chitosan, succinato de chitosan, malato de chitosan, sulfato de chitosan, acrilato de chitosan.	Rv.2 Donde la sal de chitosan comprende uno o más del grupo que comprende uno o más del grupo consistente de en: acetato de chitosan, lactato de chitosan, succinato de chitosan, malato de chitosan, sulfato de chitosan, acrilato de chitosan.
Rv.6 donde el surfactante médico consiste en uno o más del grupo que consiste en copolímeros de bloque basados en óxido de etileno y óxido de propileno.	Rv. 12 donde al menos el surfactante médico comprende uno o más del grupo que consiste en: copolímeros de bloque basados en óxido de etileno, óxido de propileno.
Rv.7 donde el surfactante médico corresponde aun ácido graso seleccionado entre ácido láurico y ácido oleico.	Rv. 13 donde al menos el surfactante médico es un ácido graso seleccionado entre ácido láurico y ácido oleico.
Rv.13 donde el polvo hemostático comprende además al menos un material inerte.	Rv. 11 donde el polvo hemostático puede comprender al menos un surfactante médico.
Rv. 14 donde el material inerte comprende uno o más del grupo que consiste en chitosán, quitina, celulosa, harina molida de maíz.	Rv. 6 donde el material inerte comprende uno o más del grupo que consiste en celulosa, sílice ahumado, arena, arcilla, alginato, goma guar, goma xantana, chitosán, derivados de chitosán, quitina, harina molida de maíz entre otros.
Rv.19 un polvo hemostático para el uso en la fabricación de una venda hemostática para heridas.	Rv.36 un polvo hemostático para el uso en la fabricación de una venda hemostática para heridas.
Rv.22 una venda hemostática para heridas en donde las fibras son de chitosan.	Rv.39 una venda hemostática para heridas en donde las fibras son de chitosan.

Por lo anterior, se sugiere al solicitante fusionar las solicitudes que se encuentran en trámite, debido a que las dos guardan el mismo concepto común inventivo. Se recuerda además al señor solicitante que la solicitud fusionada se beneficiará de la fecha de presentación y de la fecha de prioridad que corresponda a la materia contenida en las solicitudes iniciales.

4. Objeto de la invención

Titulo de la solicitud: "Material Hemostático".

El problema planteado en esta solicitud consiste en proporcionar un polvo hemostático para uso en el control de la hemorragia.

Para resolver este problema técnico la solicitud en estudio proporciona una composición conformada por una sal de chitosán junto con al menos un surfactante médico. Al menos un material inerte puede estar opcionalmente incluido.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A 61 K 31/722, A 61 L 26 / 00, A 61 L 15 / 28.

5. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 43 y 44 no es patentable de acuerdo con el Art. citado.

6. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 describe "*una sal de chitosan junto con al menos un material inerte*" la cual es una definición por la función que cumple el componente, lo que da lugar a un número ilimitado de composiciones que no encuentran soporte en la descripción. Se requiere al solicitante definir de manera especifica cuales son los componentes que hacen parte de la composición en concordancia con las realizaciones preferidas, como por ejemplo chitosan, aclarar el alcance de la reivindicación 20 por cuanto es muy similar al descrito en la reivindicación 1.

En las reivindicaciones 4, 5, 23 y 24 se establecen dos valores diferentes para la concentración de la sal de chitosan en el polvo hemostático, sin embargo, no se encuentra soporte de estos valores en la descripción de la presente solicitud, se sugiere eliminar las reivindicaciones mencionadas.

Definir para las reivindicaciones 6, 21, 25, 31 cuáles con los componentes que harán parte de la composición según las preferencias estipuladas en el campo descriptivo, por ejemplo, chitosan, ácido succìnico, àcio làurico.

Los terminos "hasta", "al menos" y "uno o màs" son relativos por lo cual no permite establecer el alcance de la invención. Se sugiere sustituirlo por un término o rango concreto.

En las reivindicaciones 8 a 10 se define la composición en términos del tamaño de partícula de sus componentes, el cual es un resultado a alcanzar ya que las composiciones se definen por sus componentes y la proporción en la que éstos se encuentran.

En las reivindicaciones 16 y 35 se define la composición en términos del pH, el cual es un resultado a alcanzar ya que las composiciones se definen por sus componentes y la proporción en la que éstos se encuentran.

En las reivindicaciones 17 a 19 se define la composición en términos de la penetrabilidad, el cual es un resultado a alcanzar ya que las composiciones se definen por sus componentes y la proporción en la que éstos se encuentran.

La reivindicación 11 no está sustentada en la descripción porque la composición con chitosan, un agente surfactante y un material inerte está presente únicamente en las reivindicaciones y no en la descripción. Se sugiere incorporarla a la descripción para

dar soporte. Esta modificación no supone ampliación puesto que las reivindicaciones iniciales se presentaron con la descripción (Art. 26 D 486).

Las reivindicación 40 carece de concisión porque define los ingredientes del proceso en términos generales como: un ácido, un solvente y un material inerte. Con la introducción de estos términos se abarca una gran variedad de componentes que deben definirse de acuerdo con la denominación común y bajo la óptica de las modalidades descritas que forman parte del soporte de la invención.

Las reivindicaciones 40 a 42 mencionan un procedimiento para la elaboración de un polvo hemostático pero este no se define mediante todas sus características técnicas esenciales como lo son las condiciones específicas como el tiempo de mezcla, condiciones de mezcla, la temperatura, la proporción de sus componentes, velocidad de adición del material inerte, entre otras variables que caractericen correctamente el proceso, se requiere caracterizar el proceso adecuadamente en concordancia con todos su pasos y características técnicas esenciales.

7. Unidad de invención (Art 25 D 486)

La Solicitud no cumple con el requisito de unidad de invención porque se refiere a 3 grupos inventivos, que aunque en todos los casos emplean quitosán como material hemostático no guardan un único concepto inventivo entre si:

Los Grupos Inventivos están definidos por las siguientes características **esenciales**:

1° Composiciones que constan de un polvo hemostático que comprende una sal de chitosán con un material inerte (Reivindicación 1). (Divulgados en D1 US5836970).
2° Composiciones que constan de un polvo hemostático que comprende una sal de chitosán con un surfactante médico (Reivindicación 12).
3. Composiciones que constan de un polvo hemostático que comprende una sal de chitosán con un surfactante médico, además un material inerte (Reivindicación 20).
4° Una venda hemostática para heridas que comprende un polvo hemostático, donde la venda comprende fibras de chitosan (reivindicación 39). (Divulgado en D3 WO2004/026200).

Se sugiere presentar las solicitudes divisionales que considere necesarias, ó limitar la solicitud inicial a una sola invención, eliminando de ella las otras invenciones.

El presente examen se llevará a cabo sobre el primer grupo inventivo definido.

8. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la fecha de Presentación de la Solicitud de Prioridad: diciembre 29 de 2005 y se encontraron los siguientes

documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	US5836970	Hemostatic wound dressing	17/11/1998
D2	Am. journal of emergency, Vol.15, N°1	A new stable pluronic ® F68 gel carrier for antibiotics in contaminated wound treatment	Ene/1997
D3	WO2004/026200	Compositions For Wound Treatment	01/04/2004

D1
http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=5836970A&KC=A&FT=D&ND=3&date=19981117&DB=EPODOC&locale=es_ES
Divulga la combinación sinérgica de la mezcla en polvo de Chitosan y un material absorbente, como el alginato, para mejorar el tiempo de curación de las heridas. (Resumen).

D2 Am. journal of emergency, Vol.15, N°1, Pág.20 a 24.
Divulga el empleo de surfactantes como portadores en el tratamiento de heridas. (Pág.23, Conclusiones, ln. 7 a 9).

D3http://worldwide.espacenet.com/publicationDetails/originalDocument?FT=D&date=20040401&DB=EPODOC&locale=es_LP&CC=WO&NR=2004026200A2&KC=A2&ND=4
Hace referencia al uso de Chitosan con un material inerte como la Celulosa Regenerada Oxidada (Pag.1, ln.16-21).

9. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 1 consiste en un polvo hemostático que comprende una sal de chitosan junto con al menos un material inerte.

Comparación de las características técnicas **esenciales**,
con las del Estado de la Técnica:

Características **esenciales**	**D1**
Polvo hemostático	Polvo Hemostático (Resumen Ln.1 a 19)
Sal de chitosan	Cloruro de chitosan (Columna 3, Ej.1)
Material Inerte	Alginato y colágeno (Columna 2, Ln.9-18)

Las características **esenciales** de la composición de la reivindicación 1 habían sido divulgadas previamente en D1, el cual da a conocer un polvo hemostático a base de una sal chitosan que contiene un material inerte representado por Alginato y colágeno (Columna 2, Ln.9-18).

En consecuencia, la reivindicación 1 no es nueva ó carece de novedad, según lo divulgado en el documento D1.

Las reivindicaciones dependientes 2 a 10 no mencionan alguna característica que

haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas.

Conclusión:
La reivindicación 1 no cumple el requisito de novedad (Art. 16).

Nivel Inventivo (Art18 D486)

La reivindicación 11 consiste en un polvo hemostático que comprende una sal de chitosan junto con al menos un material inerte y al menos un surfactante médico.

Comparación de las características técnicas **esenciales**,
con las del Estado de la Técnica:

Características **esenciales**	D1	D2
Un polvo hemostático que comprende :	Polvo Hemostático (Resumen Ln.1 a 19)	Portador de antibióticos en el tratamiento de heridas (Pág.20, resumen).
Una sal de Chitosan	Cloruro de chitosan (Columna 3, Ej.1)	
Un material inerte	Alginato y colágeno (Columna 2, Ln.9-18)	
Un surfactante médico		Un compuesto a base de bloques de copolímeros de óxido de etileno y óxido de propileno empleado como portador en composiciones para la desinfección de heridas (Pág.23, conclusión).

Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 11 porque divulgan composiciones compuestas por chitosan o sales de chitosan para el tratamiento de heridas, en especial como hemostáticos.

La diferencia entre la invención, definida en la reivindicación 1 y la composición de D1 es que en D1 no presenta un surfactante médico.

La diferencia entre la invención, definida en la reivindicación 1 y la composición de D2 consiste en que en D2 no se presentan sales de chitosan ni un material inerte.

El efecto que logra el incluir los surfactantes médicos es que se mejora la estabilidad y penetrabilidad del agente hemostático.

Por lo tanto, el Problema Técnico Objetivo que pretende resolver esta invención se puede formular así: "cómo modificar la composición conocida en D1 para conseguir una mayor estabilidad del agente hemostático y por consiguiente una eficiencia adecuada en el control de la hemorragia".

Sin embargo, la utilización de agentes surfactantes para aumentar la estabilidad y penetrabilidad de composiciones en el manejo de heridas, ya está divulgada en el documento D2 que se refiere a una composición de un agente surfactante del tipo

un agente surfactante D2 en la composición de D1 para llegar así al objeto de la reivindicación 11 de la solicitud. Por lo cual se considera obvia.

Las reivindicaciones dependientes 12 a 36 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas.

Conclusión:
La reivindicación 11 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

10. Conclusión

Los requisitos de patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US5836970	1 a 10	Novedad
D1	US5836970	11 a 36	Nivel inventivo
D2	A new stable pluronic ® F68 gel carrier for antibiotics in contaminated wound treatment	11 a 36	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante para (60) días y presentarla por escrito.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.

JESÚS FERNEL GARCÍA GARCÍA
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha 27 JUL. 2012

Elaborado por: Juan Pablo Ramos Piñeros
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A New Stable Pluronic® F68 Gel Carrier for Antibiotics in Contaminated Wound Treatment

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Pluronic® F68 was selected as the gel carrier for antimicrobial agents because of its extensive use as a wound cleanser in humans without discernable side effects. When the concentration of this surfactant is increased to 46%, it forms a water soluble gel that can serve as a carrier for antimicrobial agents. The stability of this gel can be enhanced by immediately cooling (−15°C) the gel for 24 hours before storage and subsequent application. Immediate cooling of the gel causes hydration of the surfactant that is associated with gel strengthening and prolonged shelf life stability. In experimental animals, this stable gel carrier containing 0.2% nitrofurazone significantly reduces the bacterial concentration of *Staphylococcus aureus* in wounds to a greater degree than silver sulfadiazine. This antimicrobial gel has the same antimicrobial activity as polyethylene glycol carriers containing 0.2% nitrofurazone, but does not carry the potential risk of polyethylene glycol intoxication. (Am J Emerg Med 1997;15:20-24. Copyright © 1997 by W.B. Saunders Company)

The vehicle or carrier for some antimicrobial creams or ointments have been soluble ointment bases. They are made up of soluble components or include gelled aqueous solutions.[1] The latter are often referred to as gels and in recent years have been formulated specifically to maximize drug availability. Five characteristic properties of water soluble bases include water solubility, washability, nongreasiness, nonocclusiveness, and absence of lipids.

The major components of most water soluble bases are the polyethylene glycols (PEGs).[1] These are liquids or waxy solids identified by numbers, which are an approximate indication of molecular weight. PEGs are nonvolatile and water soluble or water miscible compounds that are chemically inert with varying molecular weights from several hundred to several thousand. Patch tests have shown that these compounds are locally innocuous and continuous use has confirmed their lack of irritation.[1] The PEG, particularly with a molecular weight of 1,500, can be used as a vehicle per se; however, better results are often obtained by using blends of high and low molecular weight glycols as in PEG ointment USP. The water solubility of a PEG vehicle does not ensure availability of drugs contained in the vehicle.

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Key Words: Contaminated wound, Pluronic® F68, bacteria.

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Our initial clinical experiences with an antimicrobial cream, Furacin Soluble Dressing (FSD) (Norwich-Eaton Pharmaceuticals, Norwich, NY), in the treatment of burn patients was very favorable, especially those with minor burn injuries (5% total body surface area).[2] It could be applied easily with minimal discomfort to the patient. In addition, this antimicrobial cream could be easily removed by washing the burn wound with water. The FSD contains 0.2% nitrofurazone in a water soluble cream base. Nitrofurazone has bacteriocidal activity for a number of important burn pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter cloacae*, and *Proteus* species.[3] It does not have significant activity against *Pseudomonas aeruginosa* or fungi.

The vehicle or carrier in FSD is a blend of three PEG fractions that have average molecular weights of 300, 1,000, and 4,000. Unexplained increases in the anion gaps and serum osmolalities were observed by us in three burn patients with severe burn injury (total burn surface area 56%, 20%, and 5%) who died following treatment with FSD.[4] After other causes had been excluded, toxicity caused by absorption of polyethylene glycol was suspected. Ethylene glycol was found in the circulation and the three patients died in acute renal failure. All the patients were acidotic with increased anion and osmolal gaps. This syndrome was similar to the more common poisoning with ethylene glycol but also included an increased serum calcium with a concomitant decrease in the ionized calcium. The cause of this high "calcium gap" appeared to be binding of calcium by dicarboxylic acid metabolites of PEG. These findings were the same as those found in rabbits treated with PEG. These experimental and clinical studies have caused us to discontinue the clinical use of FSD.

We have developed a new water soluble base that is an excellent vehicle for antimicrobial agents for use in contaminated wounds. It is the purpose of this report to describe a formulation process for this new water soluble base that ensures shelf life stability and to compare the antimicrobial activity of this antimicrobial cream to FSD and silver sulfadiazine (Silvadene; Marion Merrill Dow Laboratories Inc, Kansas City, MO) in experimental animals.

MATERIALS AND METHODS

Formulation of Stable Pluronic® F68 Gel Carrier

Pluronic® polyols. Block copolymers of ethylene oxide and propylene oxide were introduced commercially in the early 1950s.[5]

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Their unique structure allowed a novel approach in the design of surface-active agents. While other nonionic surfactants have a fixed hydrophobe and can affect changes in surfactant function only by altering the hydrophile, Pluronic® copolymers allowed the alteration of both hydrophobe and hydrophile (Figure 1). Pluronic® polyols are a series of block copolymers that consist of a water-soluble poly(oxyethylene) group at both ends of a water-insoluble poly(oxypropylene) chain. The first step in synthesizing Pluronic® surfactants is the creation of a hydrophobe of desired molecular weight by the controlled addition of propylene oxide to the two hydroxyl groups of propylene glycol. The hydrophobe can then be tailored to any desired molecular weight. The customized addition of ethylene oxide sandwiches the hydrophobic base between the hydrophilic poly(oxyethylene) groups, which are controlled in length to constitute 10% to 80% (by weight) of the final molecule.

Structure toxicity studies were undertaken by our laboratory with this family of nonionic surfactants.[5] Pluronic® polyols of varying molecular weight with a wide range of ethylene oxide content were evaluated in a previous study. The effect of topical application of solutions of different Pluronic® polyols on the experimental animal wound's resistance to infection was used as a measure of toxicity. The ethylene oxide content was the most important causal factor of toxicity. Contaminated wounds treated with a polyol with a high ethylene oxide content (80% by weight of total molecule) exhibited the lowest incidence of infection. The molecular weight of the Pluronic® polyol was not a significant determinant of toxicity of these surfactants in wounds.

Pluronic® F68, a nonionic surfactant with a molecular weight of 8,350 with an ethylene oxide content constituting 80% of its molecular weight, had been previously subjected to numerous experimental and clinical studies.[6] This surfactant is not biodegradable and is rapidly excreted from the body after intravenous administration.[7] Mechanical cleansing of experimental wounds with a sponge soaked in a 20% solution of this surfactant prevented the development of infection. Treatment with Pluronic® F68 did not result in any significant damage to the cellular components of blood, wound healing, or the wound's resistance to infection. Since Pluronic® F68 was approved by the Food and Drug Administration for use in humans as a skin wound cleanser, it has been used extensively in patients without discernable toxic effects or allergic reactions.[6]

In the absence of water, Pluronic® F68 is commercially available as a solid flake. It dissolves slowly in water forming a transparent liquid solution of the surfactant. However, the Pluronic® polyols are more soluble in cold water than in hot, owing to hydrogen bonding of water with the many ether oxygen atoms.[5] As the concentration of Pluronic® F68 polyol is increased in water to 46% by weight, it forms a gel that is a two-phase colloidal system, consisting of a solid in a liquid. Its structure seems to be a network of particles linked chemically, in a continuous phase system.

Studies of gel stability. The purpose of the first part of this study was to formulate a stable gel system with a long shelf life. The gel was first prepared at room temperature (25° to 26°C). For each gel preparation, 54 mL of sterilized, deionized water was placed in a mixing bowl, after which 46 g of Pluronic® F68 was slowly added to the water. This mixture was mechanically stirred until its consistency reached that of foaming egg whites, and 0.2 g nitrofurazone was then added. Separate samples of the mixture were stored in five clear, 100-g containers at either 2°C, 7°C, 16°C, 26°C, or 33°C. During the next 120 days, each container was observed for evidence of phase transition, which is evidence of gel instability (fracture).

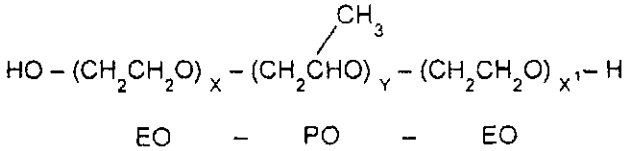

FIGURE 1. Structure of pluronic surfactants.

It was hypothesized that successful gel strengthening could be achieved by storing the mixture in cold temperatures (−15°C) before observing for evidence of phase transition at either 2°C, 7°C, 16°C, 26°C, or 33°C for 120 days or by adding 0.2 g nitrofurazone to the mixture. Gel strengthening was then associated with hydration of the surfactant by desiccating in incubators (35° to 37°C) weighed samples of the pre-cooled mixture as well as the mixture prepared at room temperature. The influence of 0.2% g nitrofurazone on gel stability was also ascertained by recording the time to phase transition for both control gels and those with antibiotics. Each sample was tested in triplicate. Statistical significance of the data was determined by the Student's *t* test using a two-tailed test with an alpha level of 0.05.

Antibacterial Activity of Stable Gel Pluronic® F68 With Nitrofurazone

Antimicrobial creams or gels. The purpose of the second part of the study was to determine the antibacterial activity in vitro and in vivo of two commercially available creams as well as the new stable Pluronic® F68 antimicrobial gel. Silver sulfadiazine cream contains 1% silver sulfadiazine in a carrier containing white petrolatum, stearyl alcohol, isopropyl myristate, sorbitan monooleate, polyoxyl 40 stearate, propylene glycol, water, and methyl paraben. FSD has 0.2% nitrofurazone in a PEG carrier (Roberts Pharmaceutical Corporation, Eatontown, NJ). The stable Pluronic® F68 gel contains 0.2% nitrofurazone.

In vitro evaluation. A standardized sensitivity test was used to evaluate the activity of the three antibacterial agents in vitro.[9] An isolation plate prepared on tryptose blood agar of *S aureus* (American Type Culture Center #12600) was prepared from a frozen aliquot maintained at −70°C using standardized microbiologic technique.

Twenty-four hours before testing, a colony from the isolation plate of *S aureus* was transferred by wire loop to an aliquot of 25 mL of trypticase soy broth. The broth bottle was agitated at 37°C for 19 hours and the bacteria were collected by centrifugation. The bacterial pellet was washed twice in 0.9% saline solution and suspended in 2 mL of 0.9% saline solution. This stock suspension was subjected to eight serial tenfold dilutions and the number of bacteria in the second diluted suspension was determined by standardized microbiologic technique.

Four confluent lawn plates were prepared from the second dilution of this suspension using standardized commercial Mueller-Hinton 15-cm agar plates having a uniform agar depth of 5 mm. Three wells were placed in each plate 4 cm from the wall of the plate and each other using a sterile plastic tube attached to vacuum. To ensure uniform wells, the same 5-mm–diameter tube was used to make all holes. Each well was then filled with one of three antimicrobial creams using a 19-gauge syringe. The plates were then incubated for 18 hours, after which the zones of inhibition were measured.

In vivo evaluation. Forty adult female Sprague-Dawley rats were anesthetized with sodium pentobarbital (43 mg/kg body weight). The hair on the dorsal surface of the animal was removed first by clipping and then depilation. After washing the depilatory from the skin, an iodophor was applied to the skin surface. A reproducible dermal wound was prepared by excising a split thickness skin graft (0.017 inch) using a dermatome. The size of the defect was approximately one square inch. An inoculum containing 10^8 *S aureus* in 0.05 mL of saline solution was applied to the wound surface using a tuberculin syringe. This inoculum was verified by

standard dilution, plating, and quantitation techniques. The wounds were then covered with a sterile nonadherent dressing. Twenty-four hours later, the animals were randomly divided into four treatment groups whose wounds received a 0.7-mL sample of either 0.9% saline (control), silver sulfadiazine, FSD, or the Pluronic® F68 antimicrobial gel. The wounds were then covered by a nonadherent dressing. On each successive day, the animals were anesthetized with halothane so that their bandages could be removed, their wounds were wiped with saline-soaked gauze sponges, and the designated treatment was reapplied, after which the animals' wounds were bandaged using aseptic technique. These treatments were repeated for 5 days, after which quantitative bacterial counts of a one-square-centimeter biopsy from each wound were performed using standardized microbiological techniques.[16] No residual antibacterial activity was detected in any of the filtered samples of the homogenates. The bacterial concentration of each biopsy was converted to its base 10 logarithm and the results for each treatment group were expressed as the mean and standard deviation. Statistical analysis of the data was determined by a two-tailed Student's *t* test using an alpha level of 0.05 for significant difference. All scientific protocols were reviewed and approved by the Animal Research Committee of the University of Virginia.

RESULTS

Pluronic® F68 gels prepared at room temperature (25°C) were not stable and underwent phase transition after 4 months of storage at 7°C, 16°C, 26°C, and 33°C (Table 1). Pluronic® F68 gels stored at 2°C were the most susceptible to phase transition, which was evident within 15 minutes after preparation.

Preparation and storage of the Pluronic® F68 gels in a cold environment (−15°C) for 24 hours strengthened the gels and prolonged their shelf life stability ($P < .01$) (Table 2). These precooled Pluronic® F68 gels showed no evidence of phase transition during a 4-month period of storage at temperatures of 2°C, 7°C, 16°C, 26°C, and 33°C.

The Pluronic® F68 gel strengthening was related to hydration of the surfactant (Figure 2). When the Pluronic® F68 gels were prepared at cold temperatures (−15°C) or room temperature (25°C) there were notable differences in their weights after desiccation. The weight of the Pluronic® F68 gels prepared at cold temperatures remained significantly higher than those prepared at room temperature after 11 and 15 days of desiccation ($P < .05$). The increased weight of the Pluronic® F68 gels prepared at cold temperatures is attributed to hydration of the surfactant.

The presence of an antibiotic in the Pluronic® F68 gel did not increase its stability. In Pluronic® F68 gels prepared at room temperature, the time to phase transition for control gels and those with 0.2% nitrofurazone was only 15 minutes.

TABLE 1. Pluronic® F68 Prepared at 25°C and Stored at Different Temperatures

°C	1 Day	1 Week	1 Month	4 Months
2	U	U	U	U
7	S	S	S	U
16	S	S	S	U
26	S	S	S	U
33	S	S	S	U

ABBREVIATIONS: S, stable; U, unstable.

TABLE 2. Pluronic® F68 Prepared at −15°C and Stored at Different Temperatures

°C	1 Day	1 Week	1 Month	4 Months
2	S	S	S	S
7	S	S	S	S
16	S	S	S	S
26	S	S	S	S
33	S	S	S	S

ABBREVIATIONS: S, stable; U, unstable.

All antimicrobial creams had demonstrable antibacterial activity. However, the gels or creams containing nitrofurazone exhibited a significantly greater antibacterial activity than did silver sulfadiazine in the in vitro and in vivo evaluations. The zones of inhibition for the nitrofurazone Pluronic® F68 gel (32 ± 4 mm) and FSD (34 ± 5 mm) did not differ significantly, but were significantly greater than those encountered with silver sulfadiazine (14 ± 5 mm) ($P < .05$). Similarly, the superior antimicrobial action of the nitrofurazone gels or creams over silver sulfadiazine was apparent in contaminated wounds. The mean log of bacterial concentration following treatment with nitrofurazone gels or creams was significantly less than that after treatment with silver sulfadiazine ($P < .01$) (Figure 3). The mean log of bacterial concentrations of the contaminated wounds after treatment with the creams or gels containing nitrofurazone did not differ significantly. It is important to emphasize that the mean log of bacterial concentration of the contaminated wounds subjected to each of the three different antibacterial agents were significantly less than that encountered in the controls ($P < .01$).

Another notable advantage of the nitrofurazone creams or gels was that their water soluble bases make them easy to apply and to remove. In contrast, the silver sulfadiazine aggressively adheres to the tissue and requires extensive rinsing and mechanical removal with sponges before reapplication.

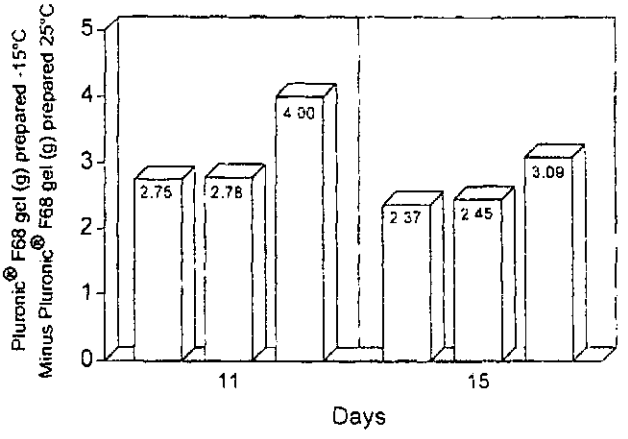

FIGURE 2. After desiccation for 11 to 15 days, the weight of the Pluronic® F68 gel prepared at cold temperatures (−15°C) was significantly greater than that of Pluronic® F68 gels prepared at room temperature (25°C) suggesting that the surfactant becomes hydrated at low temperatures.

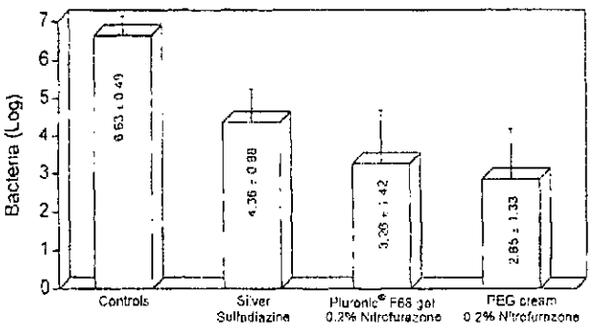

FIGURE 3. Nitrofurazone creams or gels significantly reduced the bacterial concentration of contaminated experimental wounds to a greater degree than did silver sulfadiazine.

DISCUSSION

The ideal antimicrobial cream or gel for topical use should have several important characteristics. It should have a broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria as well as fungal species. It should be easy to apply to the wound, with minimal pain to the patient. In addition, it should be water soluble so that it can easily be removed from the wound, facilitating reapplication. It should be able to penetrate viable and nonviable tissue. It must have no discernable adverse local or systemic effects. It must be easily stored, with a long shelf life.

Today, there is no commercially available agent that meets all of these criteria. Silver sulfadiazine is currently the most widely used topical antimicrobial agent. It has a wide antimicrobial spectrum, although little is known about its mechanisms of action and the respective roles of the silver and sulfonamide components. It is minimally absorbed into the eschar, although there is some absorption of sulfonamide into the bloodstream.[11] The most common adverse effect encountered clinically with the use of silver sulfadiazine is transient leukopenia.[12] The leukopenia typically appears 2 to 3 days after initiation of therapy and is associated with a disproportionate decrease in the neutrophil population. Despite a 5% to 15% incidence of this phenomena there have been no reports of an increased incidence of infectious complications.[13] Cutaneous sensitivity reactions are typically a macular papular rash, occur in less than 5% of patients, and rarely require stopping the use of silver sulfadiazine.[3]

The repeated use of silver sulfadiazine in major burns was associated with significant increases in serum osmolality.[14,15] Investigation of the composition of the cream reveals that it contains the low-molecular-weight glycol, propylene glycol (15%). The serum osmolality gap in these patients was correlated with and accounted for by the measured concentrations of propylene glycol in the serum. In addition, the high anion gaps in these patients were explained by increased concentrations of the metabolic product of propylene glycol, lactic acid; however, the propylene glycol and its metabolic product, lactic acid, were not reported to produce organ toxicity.

Unlike propylene glycol, topical application of PEG causes severe systemic toxicity, which is characterized by elevated total calcium, elevated osmolality gap, high anion gap metabolic acidosis, and renal failure.[16] We believe that the PEG metabolites produce renal destruction via mechanisms similar to those involved in the renal failure associated with ethylene glycol poisoning. This new nitrofurazone Pluronic® F68 gel appears to have distinct advantages over silver sulfadiazine and FSD. It is a water soluble base that can be easily applied and removed from the contaminated wound by rinsing with sterile saline. In addition, it exhibits superior antimicrobial activity as compared to silver sulfadiazine. The hydrated form of this gel is very stable and exhibits a long shelf life. This observation that hydration of Pluronic® F68 maintains gel stability is consistent with the results of other studies of PEG complexes. A trihydrate could contain as much as 42 moles of water without destroying structure integrity while a monohydrate could contain only 3 moles.[17]

Pluronic® F68 water soluble base has been used extensively in humans without discernable toxic effects. The only drawback to the use of this new gel is the limited activity of nitrofurazone against *Pseudomonas* species. This deficiency can be easily corrected by adding polymyxin to the gel.

CONCLUSION

It was the purpose of this report to describe a formulation process for a water soluble base, Pluronic® F68, that ensures shelf life stability and to compare the antimicrobial activity of this antimicrobial gel to FSD and silver sulfadiazine in experimental animals. In the absence of water this surfactant is a solid flake. The addition of water to Pluronic® F68 results in a transparent solution. When the concentration of the surfactant reaches 46%, a gel is formed that can serve as a carrier for the antimicrobial agents. The stability of the gel can be enhanced by preparing the gel at cold temperatures (−15°C). Preparation of the gel in a cold environment hydrates the surfactant, which enhances the stability of the gel.

This water soluble base is an effective carrier of nitrofurazone for use in contaminated wounds. Using quantitative bacterial culture techniques, it has been demonstrated that Pluronic® F68 nitrofurazone gel suppresses the bacterial concentration of contaminated wounds to a greater degree than silver sulfadiazine. Its antimicrobial activity is comparable to that of FSD without the dangers of systemic toxicity.

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