



Industria y Comercio
SUPERINTENDENCIA

SOLICITUD
PATENTE DE INVENCION

21 EXPEDIENTE N°

03-29592

54 TÍTULO "USO DE ARGININA EN LA PREPARACION DE UN MEDICAMENTO PARA LA PREVENCION

Y EL TRATAMIENTO DE LOS EFECTOS COLATERALES ASOCIADOS CON LA ADMINISTRA-
CION ENDOVENOSA DE PRODUCTOS FARMACEUTICOS"

51 CLASIFICACIÓN INTERNACIONAL A61K 31/66

71. SOLICITANTE PHARMACIA ITALIA S.P.A.

DOMICILIO MILAN, ITALIA

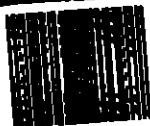
74. REPRESENTANTE LEGAL JOSE ANTONIO LLOREDA

T.P. 10.784
Código 231

22 BOGOTÁ, D.C. 8 ABR. 2003

PETITORIO

Industria y Comercio
SUPERINTENDENCIA 03 028592 00



SUPERINDUSTRIA Y COMERCIO
Radicación : 83029592 00000000 Folios: 128
Fecha (AMB): 2003-04-08 16:12:25
Trámite : T-1 PCT-PATENT 379 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

**FORMULARIO UNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL**

①

SOLICITUD DE:



Patente de Invención



Patente de Modelo de Utilidad



Capítulo I.



Capítulo II.

②

SOLICITANTE
(71)

Nombre: PHARMACIA ITALIA S.p.A.

Dirección: Via Robert Koch, 1.2
I-20152 Milan, Italia.

Nacionalidad o Domicilio: Milan, Italia

Lugar de Constitución: Italia

Teléfono: 3284270 Fax: 6069701

E-mail: jllcco@lloredacamacho.com

IDENTIFICACION

C.E.



Otro



C.C.



NIT



Cual _____

Número _____

③

REPRESENTANTE
O APODERADO

Nombre: JOSE ANTONIO LLOREDA

Dirección: Calle 72 No. 5-83 Piso 5o.

Teléfono: 3284270 Fax: 6069701

E-mail: jllcco@lloredacamacho.com

C.C.



NIT



C.E.



Otro



Cual _____

Número 17.159.881 TP 10.784

④

INVENTOR(ES)
(72)

Nombre: MUGGETTI, Lorena; MARTINI, Alessandro; BUZZI, Giovanni; COLOMBO, Paolo.

Dirección: Via Trento, 38, I-20036 Meda; Via Desiderio da Settignano, 14, I-20149 Milan; Via F. Poggi, 14, I-20131 Milan; Via Ludovico Cavaleri, 3, I-20147 Milan.

Nacionalidad o Domicilio: ITALIA

⑤

PCT (86)

Solicitud Internacional No. PCT/EP01/10398

Fecha: Septiembre 07, 2001

Publicación Internacional No. WO 02/22134 A1

Fecha: Marzo 21, 2002

⑥

Título (54) "USO DE ARGININA EN LA PREPARACION DE UN MEDICAMENTO PARA LA PREVENCION Y EL TRATAMIENTO DE LOS EFECTOS COLATERALES ASOCIADOS CON LA ADMINISTRACION ENDOVENOSA DE PRODUCTOS FARMACEUTICOS"

⑦

Clasificación Internacional (51) A61K 31/86

⑧

Prioridad

SI



NO



(33) País de Origen

IT

(32) Fecha

12-09-00

(31) Número de Solicitud

MI2000A001984

⑨

Comprobante de Pago No. 02-86,486 y 03-15,527

Fecha: Diciembre 24, 2002 y Marzo 4, 2003

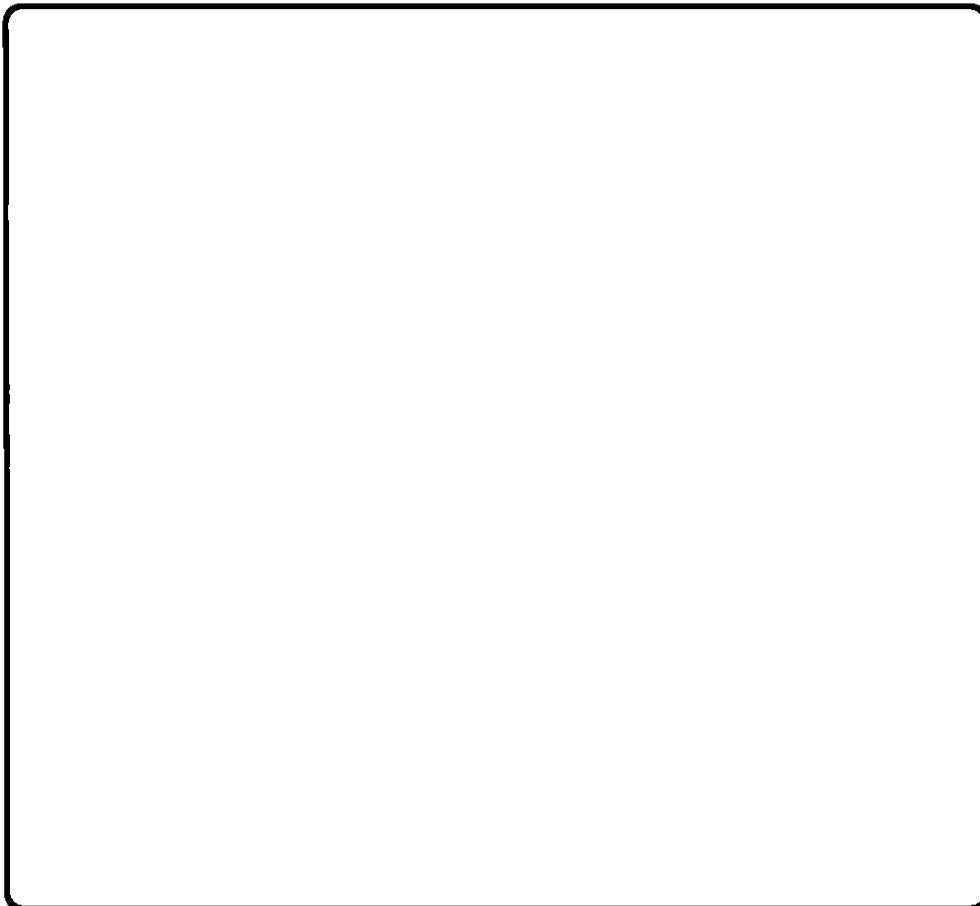
ANEXOS

10

- ☒ Comprobante de pago de la tasa de presentación de la solicitud.
- ☒ Documento que acredite la existencia y representación legal cuando el solicitante sea persona jurídica.
- ☒ Documento de Representación Legal.
- ☐ Documento de cesión del inventor al solicitante o a su causante.
- ☐ Declaraciones
- ☐ Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones)
- ☒ Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).
- ☒ Traducción del examen preliminar internacional efectuado por la IPEA.
- ☐ De ser el caso, copia del contrato de acceso.
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- ☐ Arte Final 12X12.
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionados parcial o totalmente con la invención de esta solicitud.
- ☐ De ser el caso, modificaciones efectuadas a la solicitud internacional.
- ☒ Otros: Reporte Búsqueda Internacional y su traducción, Anexos Reporte de Búsqueda Internacional, Publicación con su traducción y Notificación del Registro de un cambio de nombre y su traducción.

11

FIGURA CARACTERISTICA:



12

Solicito la concesión de la patente.

NOMBRE: JOSE ANTONIO LOREDA

FIRMA:

C.C. 17.150.687 BA. T.P. 10.764



Industria y Comercio
SUPERINTENDENCIA

SUPERINDUSTRIA Y COMERCIO
Radicacion : 83825532 00000030 Folios: 126
NIT : 800176089-2
Fecha (AMD): 2003-04-06 16:42:25
Tramite : 011 PCT-PATENT 379 FASE NNC1 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 02 - 86,486
FECHA : DICIEMBRE 24 DE 2002

***** CONSIGNACION *****

DEPOSITANTE	TIPO PASO	BANCO	CUENTA	No. PASO	FECHA PASO	Vr. PASO
LLORERA Y CIA S. A	CONSIGNACION	BANCO POPULAR	050-00110-6	3139931	24/12/2002	149,623,700.00

***** CONCEPTO *****

CANT. RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1 50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	378,000.00
	TOTAL :	\$ 378,000.00

SOM: TRESCIENTOS SETENTA Y OCHO MIL PESOS

RESPONSABLE : 

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

Carrera 13 No. 27-00 Pisos 5, 7 y 10
Conmutador 382 0840
Fax 350 5220
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia


Industria y Comercio
SUPERINTENDENCIA
SUPERINDUSTRIA Y COMERCIO

Radicacion : 83829592 00000002

Folios: 128

Fecha (MM): 2003-04-03 16:42:25

Trámite : 011 PCT-PATENT 379 FASE NNCI 411 PRESENTAC

Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

NIT : 800176089-2

 CERTIFICACION DE EXCEDENTE : 03 - 15,527
 FECHA : MARZO 4 DE 2003

******* CONSIGNACION *******

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
LLOREDA Y CIA S. A	CONSIGNACION	BANCO POPULAR	050-00110-6	8284110	04/03/2003	345,000.00

******* C O N C E P T O *******

CANT. RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1 50005-01-09 OTROS SERVICIOS DE PROPIEDAD I	99 OTROS SERVICIOS ADMINISTRATIVOS	22,000.00
	TOTAL :	\$ 22,000.00

SON: VEINTIDOS MIL PESOS

RESPONSABLE :

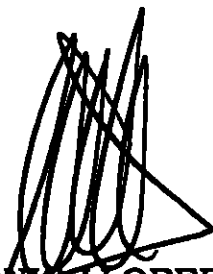
RECIBO DE CAJA APLICADO AL EXPEDIENTE No.

 ESTA CERTIFICACION DE EXCEDENTE DE PAGO SOLO PODRA SER UTILIZADA
 COMO PARTE DE LA CANCELACION DE UNA TARIFA VIGENTE Y NO PODRA SER
 SUSTITUIDA POR NINGUN RECIBO.

 Cageta 13 No 77-00 Pisos 5, 7 y 10
 Correo Electrónico: info@sic.gov.co
 Correo Fijo: 352 0840
 Fax 350 5220
 E-mail: info@sic.gov.co
 www.sic.gov.co
 Bogotá, D.C., Colombia

ANEXO

El documento que acredita la calidad de REPRESENTANTES LEGALES en Bogotá de los doctores **JOSE ANTONIO LLOREDA** y/o **GUSTAVO TAMAYO** y/o **ANGELA MARIA RESTREPO** y/o **ALICIA LLOREDA** para TODOS LOS ASUNTOS RELACIONADOS CON LA PROPIEDAD INDUSTRIAL de la sociedad peticionaria, que faculta a cada uno de dichos abogados para actuar individualmente, se esta presentando con esta solicitud. En consecuencia, ruego a usted reconocer la personería de cada uno de dichos Representantes Legales para actuar dentro del presente expediente, a fin de que cualquiera de ellos pueda intervenir en cualquier tiempo respecto de la solicitud en él contenida.

**JOSE ANTONIO LLOREDA****T.P. No. 10.784****Código 231**

TRADUCCION OFICIAL DE UN DOCUMENTO ESCRITO EN ITALIANO
(traducción de sellos)

(Apostille n. 2055)

Dra. GRAZIA SCARPONI
NOTARIO
20123 MILAN - Via Stampa, n. 15.
Codigo Fiscal : SCB GRZ 38R47 H501V

AUTENTICACION DE FIRMA

== Certifico yo suscrita Dra. GRAZIA SCARPONI, Notario en Milán e inscrita al Colegio Notarial de Milán, verdadera y auténtica la firma puesta al respaldo y en mi presencia, previa renuncia con mi consentimiento a la asistencia de testigos, de la Señora VERONELLI PAOLA, nacida en Milán el 17 de diciembre de 1945 y domiciliada al efecto en la sede social, empleada, en su calidad de apoderado de la Sociedad "PHARMACIA ITALIA S.p.A." con sede en Milán, Via Robert Koch n. 1.2, investida de los necesarios poderes.

De su identidad personal, calidad y poderes yo Notario estoy segura.

Declaro, además, yo Notario, de conocer el idioma inglés y que el texto en italiano corresponde al texto en inglés.

== Milán, 13 de marzo de 2002.

(fdo.) Grazia Scarponi, Notario (hay sello)

" APOSTILLE "

(Convención de La Haya del 5 de octubre de 1961)

1. País :

ITALIA

el presente acto público

2. ha sido firmado por Grazia Scarponi,
3. Agente en calidad de Notario en Milán,
4. esta' refrendado con el sello notarial,

certificado

5. en Milán..... - 6. El 18 de marzo de 2002,
7. por la oficina del Ministerio Público,
8. bajo el número 2055 AP,
9. y esta' refrendado con el sello de Estado.

===== **EL SUSTITUTO PROCURADOR DE LA REPUBLICA**
(fdo.) Dra. Ada Rizzi. (hay selo) -

MINISTERIO DE RELACIONES
EXTERIORES
444287
Domenico Cioffoni
COYA FIRMA APARECE EN EL
DOCUMENTO SE PENA
LAS FUNCIONES INDICADAS



Domenico Cioffoni
c.c. 760.006-5
(18 MARZO 2002)
Pec 04/006-5

ITALIA
Los abajo firmados, PHARMACIA S.P.A., una sociedad organizada y existente de conformidad con las leyes de Italia,

con domicilio en

Milán Via Robert Koch, 1.2., Italia,

nombramos por el presente a JOSE ANTONIO LLOREDA y/o GUSTAVO TAMAYO y/o ANGELA MARIA RESTREPO y/o ALICIA LLOREDA, domiciliados en Bogotá, Colombia, con oficinas en dicha ciudad, Calle 72 No. 5-83, piso 8º., nuestros representantes legales y verdaderos en Bogotá, con facultades para recibir notificaciones y nombrar apoderados judiciales o extrajudiciales, con el fin de dar cumplimiento al parágrafo 1o. del artículo 543 del decreto 410 de 1971.

Los doctores JOSE ANTONIO LLOREDA y/o GUSTAVO TAMAYO y/o ANGELA MARIA RESTREPO y/o ALICIA LLOREDA, o los apoderados que cualquiera de ellos designe, quedan especialmente facultados para representarnos ante las autoridades colombianas, administrativas, judiciales o de policía, en todos los trámites relativos a la propiedad industrial, a cuyo efecto les facultamos para dar ante dichas autoridades todos los pasos necesarios al objeto indicado; elevar solicitudes, declaraciones, reclamos y recursos; formular descripciones, enmiendas, oposiciones y apelaciones; instaurar cancelaciones y amparos administrativos; abonar todos los impuestos y cuotas y efectuar todo otro pago determinado por la ley; recibir todos los documentos y valores, dando el descargo respectivo; llenar cualesquiera otros requisitos y tomar, en fin, todas las medidas que creyeren conducentes al resguardo de nuestros intereses; y en caso de producirse oposición, pasando los antecedentes a los tribunales, quedan facultados para tomar intervención como demandantes o demandados ante los jueces que sean competentes, pudiendo transar, someter a árbitros, desistir, percibir y apelar, con todas las demás facultades que resulten necesarias, y por el presente declaramos desde ahora válido y bueno cuanto dichos representantes y/o los apoderados que cualquiera de ellos nombre hicieren en nuestro beneficio.

Asimismo, facultamos a los doctores JOSE ANTONIO LLOREDA y/o GUSTAVO TAMAYO y/o ANGELA MARIA RESTREPO y/o ALICIA LLOREDA, para solicitar en la Oficina de Propiedad Industrial la protocolización de este documento y de cualquier poder que cualquiera de ellos otorgue a nombre nuestro.

Dado y firmado Milan, Marzo 13 de 2002

The undersigned, PHARMACIA ITALIA S.P.A., a corporation organized and existing under the laws of

Italy

of

Milan, Via Robert Koch, 1.2
Italy,

hereby appoint, JOSE ANTONIO LLOREDA and/or GUSTAVO TAMAYO and/or ANGELA MARIA RESTREPO and/or ALICIA LLOREDA, of Bogota, Colombia, with offices in said town, Calle 72 No. 5-83, Sixth Floor, our true and legal representatives in Bogotá, with power to receive notifications and to appoint judicial or extrajudicial agents, so as to comply with paragraph 1o. of article 543 of decree 410 of 1971.

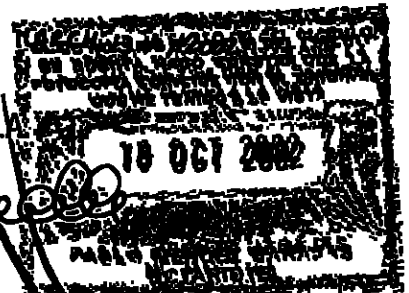
Doctors JOSE ANTONIO LLOREDA and/or GUSTAVO TAMAYO and/or ANGELA MARIA RESTREPO and/or ALICIA LLOREDA, or the agents by any one of them appointed, are especially empowered to represent us before the administrative, judicial or police authorities of Colombia, in all proceedings pertaining to Industrial property, for which purpose we authorize them to take before the said authorities all the necessary steps for the said end; to file petitions, declarations, claims, recourses, cancellations and administrative injunction proceedings; to draw up specifications; to file amendments, oppositions and appeals, to pay all installments and effect all other payments prescribed by law; to receive proper receipts, to comply with all other requirements and, in short, to take all those measures which they may deem fit for the protection of our interests; and in case of opposition, where the proceedings be passed over to the courts, we authorize said agents to act on our behalf, as complainants as well as defendants, before the competent judges, with full powers to make arrangements, to submit to arbitrators, to desist, to collect, to appeal and, in short, with all such further powers as may be required, and we hereby declare from now on as valid and good all that said representatives and/or the agents by any one of them appointed may do for our benefit.

We also authorize doctors JOSE ANTONIO LLOREDA and/or GUSTAVO TAMAYO and/or ANGELA MARIA RESTREPO and/or ALICIA LLOREDA, to apply for protocolization of this document and of any power of attorney by any one of them conferred in our name, before the Office of Industrial Property.

Given and signed this MILAN,

PHARMACIA ITALIA S.p.A.

Paola Restrepo
Executive Officer



2055 11p

Dott. GRAZIA SCARPONI
NOTAIO
20128 MILANO - Via Stampa, 18
Cod. F. 8CR GRZ 28947 H801V

AUTENTICA DI FIRMA

Certifico io sottoscritta Dott. GRAZIA SCARPONI, Notaio in Milano ed iscritta presso il Collegio Notarile di Milano, vera ed autentica la firma retro apposta in mia presenza, previa rinunzia con il mio consenso all'assistenza dei testimoni, dalla Signora VERONELLI PAOLA, nata a Milano il 17 dicembre 1945 e domiciliata per la carica presso la sede sociale, impiegata, nella sua qualità di procuratore della Società "PHARMACIA ITALIA S.p.A." con sede in Milano-Via Robert Koch 1.2, munita degli occorrenti poteri.

Della sua identità personale, qualifica e poteri io Notaio sono certa.

Dichiaro, inoltre, io Notaio di conoscere la lingua inglese e che il testo in italiano corrisponde al testo in inglese.

Milano, li 13 MAR. 2002



Grazia Scarponi

APOSTILLE

Il presente atto è stato redatto il 5 ottobre 1991
in presenza di PAOLA

Il presente atto pubblico
è stato sottoscritto da *Grazia Scarponi*
nella sua qualità di *Notaio in Milano*
con il consenso/timbro di
Sig.lla notabile

Attestato

Il Notaio il 13 MAR 2002

Il presente atto è stato redatto il 5 ottobre 1991

2055 11p

Sig.lla di Paola

Paola

pubblica
(Dott.ssa Anna Nizzi)



NOTARIAL CERTIFICATE (INDIVIDUAL)

CERTIFICADO NOTARIAL (INDIVIDUO)

{ss

{ss

A los días del mes de de 20
compareció personalmente ante mí, Notario Público
en y por el condado antes citado,

a quien doy fe de conocer y me consta que
es/son la persona descrita y firmante del documento que
precede, declarando ante mí que otorga el mismo para los
fines y propósitos que en él se expresan.

Notary Public (Notario Público).

On this day of , 20 , personally
appeared before me, a Notary Public, in and for the above
mentioned county,
PAOLA VERONELLI
to me known to be the person described in and
who executed the foregoing document, and who duly
acknowledged to me that executed same for the
uses and purposes therein expressed.

NOTARIAL CERTIFICATE (INDIVIDUAL)

{ss

{ss

A los días del mes de de 20
compareció personalmente ante mí,
Notario Público en y por el condado antes citado,

a quien doy fe de conocer y me consta que
es/son la persona descrita y firmante del documento que
precede, declarando ante mí que otorga el mismo para los
fines y propósitos que en él se expresan.

Notary Public (Notario Público).

On this day of , 20 , personally
appeared before me, a Notary Public, in and for the above
mentioned county,
PAOLA VERONELLI
to me known to be the person described in and who
executed the foregoing document, and who duly
acknowledged to me that executed same for the
uses and purposes therein expressed.

NOTARIAL CERTIFICATE (CORPORATION)

{ss

{ss

Hoy 13 de Marzo de 2002, compareció
PAOLA VERONELLI a quien conozco y
de quien me consta que firmó el anterior documento, y
quien debidamente juramentado declaró y dijo que él es
Funcionaria de PHARMACIA ITALIA S.P.A.,
la compañía nombrada en el documento que antecede,
una sociedad debidamente organizada y existente
conforme a las leyes de Italia, domiciliada en la
ciudad de Milan, legalmente constituida para
un período de duración que aún no ha terminado, que
ejerce legalmente su objeto conforme a las leyes de
que el declarante está autorizado por dicha Compañía
para conferir este poder; que el sello estampado por él al
pie de tal poder, en nombre y en representación de la
expresada Compañía, es el sello oficial de ella; y que el
objeto para el cual se confiere este instrumento está
comprendido dentro del de la sociedad.

El suscrito Notario ha visto los Estatutos de Incorporación
firmados en Milan

, con fecha , y certifica
que de conformidad con la legislación de dicho Estado la
anterior declaración juramentada es prueba suficiente de
que los hechos sobre que ella versa son ciertos o
verdaderos, de lo cual da fe.

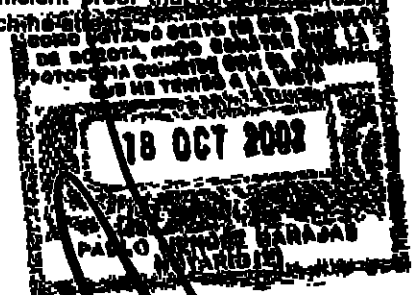
Notary Public (Notario Público).

María Amparo Molino

On this 13 day of March , 2002
personally
came **PAOLA VERONELLI**
to me known, and known to me to be the person who
signed the foregoing instrument, who being by me duly
sworn did depose and say that he is the **ITALIA**
EXECUTIVE OFFICER of **PHARMACIA S.P.A.**, the
company named within, a Corporation organized and
existing under the laws of **ITALY**, domiciled at
the City of **MILAN**, legally constituted for
a period of tenure not yet expired, which exercises its
corporate purposes according to the laws of
; that the deponent is authorized by said Company to
confer this power of attorney; that the seal affixed
hereunto by the deponent in the name and on behalf of
said Corporation is the corporate seal thereof; and that the
purpose for which this instrument is granted is included
within the corporate purposes of the Company.

The undersigned Notary Public has seen the articles of
Incorporation of said Corporation executed at
MILAN, under date of

, and hereby
certifies that under the laws of said State the foregoing
acknowledgment is sufficient proof that the facts therein
contained are true, which he attests under the seal of his
office.



Folio 8 Extracto de Publicación
Folio 9 Tarjeta Autor
Folio 10 Tarjeta Temática

REPORTE DE BUSQUEDA INTERNACIONAL

Solicitud Internacional No.
PCT/EP 01/10398

A. CLASIFICACION DE MATERIA IPC 7 A61K31/66 A61K31/565 A61K31/704 A61P35/00 A61K9/08 A61K47/18 Según la Clasificación de Patentes Internacional (CPI) o según la clasificación nacional y la CPI		
B. CAMPOS BUSCADOS Documentación mínima buscada (sistema de clasificación seguido de símbolos de clasificación) IPC 7 A61K Documentación buscada fuera de documentación mínima hasta donde dichos documentos se incluyan en los campos buscados Base de datos electrónica consultada durante la búsqueda internacional (nombre de la base de datos y, donde sea práctico, buscar términos usados) EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, PASCAL, EMBASE, CHEM ABS Data		
C. DOCUMENTOS CONSIDERADOS RELEVANTES		
Categoría *	Citación de documento, con indicación, donde sea apropiado, de los textos relevantes	Relevante a la reivindicación No.
P, X	WO 01 19372 A (COLOMBO PAOLO; BUZZI GIOVANNI (IT); MUGGETTI LORENA (IT); MARTINI) Marzo 22, 2001 (2001-03-22) citado en la solicitud el documento completo ---	1-11, 19
A	US 5 780 446 A (RAMU AVNER) Julio 14, 1998 (1998-07-14) columna 1, línea 48 - línea 58 ---	1-11, 19
☐ En el recuadro C se enumeran más documentos. ☒ En el anexo se enumeran miembros de la familia de patentes.		
* Categorías especiales de documentos citados: 'A' documento que define el estado general de la técnica el cual no se considera de relevancia particular 'E' documento anterior pero publicado en o después de la fecha de presentación internacional 'L' documento que puede arrojar dudas sobre reivindicaciones de prioridad o que se cita para establecer la fecha de publicación de otra citación u otra razón especial (según se especifica) 'O' documento que se refiere a una revelación oral, uso, exhibición u otros medios 'P' documento publicado antes de la fecha de presentación internacional pero después de la fecha de prioridad reivindicada 'T' documento posterior publicado después de la fecha de presentación internacional o fecha de prioridad y que no está en conflicto con la solicitud pero citado para entender el principio o teoría que sustenta la invención 'X' documento de relevancia particular; la invención reivindicada no puede considerarse nueva o no puede considerarse que comprende un paso inventivo cuando el documento se toma solo 'Y' documento de relevancia particular; la invención reivindicada no puede considerarse que comprende un paso inventivo cuando el documento se combina con uno o más documentos, siendo dicha combinación obvia para una persona experta en la técnica 'Z' documento miembro de la misma familia de patentes		
Fecha de la terminación real de la búsqueda internacional Febrero 8 de 2002		Fecha de envío por correo del reporte de búsqueda internacional 26/02/2002
Nombre y dirección de correo de ISA/US European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. No. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax No. (+31-70) 340-3016		Funcionario autorizado Zimmer, B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/10398

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/66 A61K31/565 A61K31/704 A61P35/00 A61K9/08
A61K47/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, PASCAL, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 01 19372 A (COLOMBO PAOLO ;BUZZI GIOVANNI (IT); MUGGETTI LORENA (IT); MARTINI) 22 March 2001 (2001-03-22) cited in the application the whole document	1-11,19
A	US 5 780 446 A (RAMU AVNER) 14 July 1998 (1998-07-14) column 1, line 48 - line 58	1-11,19

☐ Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document not published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

Date of the actual completion of the international search

8 February 2002

Date of mailing of the international search report

26/02/2002

Name and mailing address of the ISA

European Patent Office, P.O. 5818 Patentkan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-3040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3018

Authorized officer

Zimmer, B

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 01/10398

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0119372 A	22-03-2001	AU WO 7776200 A 0119372 A1	17-04-2001 22-03-2001
US 5780446 A	14-07-1998	AU WO 3656397 A 9801136 A1	02-02-1998 15-01-1998

**DOCUMENTOS ANEXOS AL REPORTE DE
BUSQUEDA INTERNACIONAL**

1. WO 01 19372 A
2. US 5 780 446 A

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number
WO 01/19372 A1

(51) International Patent Classification⁷: A61K 31/565,
31/66, 9/08, A61P 35/00, A61K 47/18

(21) International Application Number: PCT/EP00/08983

(22) International Filing Date:
13 September 2000 (13.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9921960.2 16 September 1999 (16.09.1999) GB

(71) Applicant (*for all designated States except US*): PHAR-
MACIA & UPJOHN S.P.A. [IT/IT]; Via Robert Koch, 1.2,
I-20152 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): MUGGETTI,
Lorena [IT/IT]; Via Trento, 38, I-20036 Meda (IT).
COLOMBO, Paolo [IT/IT]; Via Ludovico Cavaleri, 3,
I-20147 Milan (IT). MARTINI, Alessandro [IT/IT]; Via
Desiderio da Setignano, 14, I-20149 Milan (IT). BUZZI,
Giovanni [IT/IT]; Via F. Poggi, 14, I-20131 Milan (IT).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- With international search report.
- Before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments.

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*



WO 01/19372 A1

(54) Title: FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE AND AMINO ACIDS

(57) Abstract: A pharmaceutical formulations which comprises a parenterally acceptable carrier or diluent and estramustine phos-
phate and a basic amino acid. The formulation can be administered according to a combined chemotherapy regimen in association
with one or more chemotherapeutic agents. The formulation also enables the estramustine phosphate to be administered with no side
effects at the site of injection.

**FORMULATIONS FOR PARENTERAL USE OF ESTRAMUSTINE PHOSPHATE
AND AMINO ACIDS**

5

The present invention relates to pharmaceutical formulations of estramustine phosphate for parenteral use and, more particularly, to formulations of estramustine phosphate for parenteral use further comprising basic amino acids.

10

Estramustine phosphate (The Merck Index, XII Ed., No. 3749, 1996) is an estradiol-17 β -phosphate derivative widely known in the art as antitumor agent, currently used in the treatment of advanced adenocarcinoma of the prostate.

15

The drug is usually administered orally, preferably at a dose of 10-15 mg/kg/day. Intravenous administration, however, is also adopted in some particular cases.

For example, initial intravenous administration of estramustine phosphate, followed by oral administration, has been reported at dosages paralleling the oral administration for the drug, i.e. 300-600 mg daily given intravenously and usually repetitively over for several consecutive days (see, for a reference, British Journal of Urology, 1977, 49, 73-79; J. Urol. 108:303-306, 1972; Eur. Clin. Pharmacol. 26(1), 113-119, 1984; Eur. Urol. 1990, 17, 216-218).

20

25

Estramustine phosphate as well as other well-known cytotoxic compounds used in antitumor therapy are known to cause, or potentially cause, vascular damages at the site of injection when parenterally, in particular intravenously, administered.

30

As an example, studies in patients treated with estramustine phosphate administered as a slow intravenous injection or as a bolus, at 300 mg/day, revealed

35

9

thrombophlebitis and local irritations at the peripheral intravenous injection sites.

These drawbacks are considered major limitations for the intravenous administration of estramustine phosphate, thus requiring, in many patients, the establishment of central line administration or, in some cases, even discontinuation of the treatment.

With the aim of minimising the unwanted effects associated with the intravenous administration of cytotoxic agents, a few means are reported in the art.

Among them is the use of cyclodextrins, for instance hydroxypropyl-cyclodextrin, in the preparation of formulations for parenteral administration of cytotoxic known to cause ulcerative lesions. See, for a reference, US patent No. 5,804,568 in the name of Supergen Inc.

Also known in the art are formulations for the intravenous administration of estramustine phosphate containing human albumin, reported to be characterised by fewer local side-effects upon injection of the active (see, for a reference, H. Schutz et al.; Krankenhauspharmazie, II year, issue No. 3, 1988).

In this respect, we found formulations for parenteral use comprising estramustine phosphate together with a basic amino acid which, unexpectedly, resulted to achieve optimal protection from side-effects associated with estramustine administration.

It is therefore the object of the present invention a formulation for parenteral use comprising estramustine phosphate and a basic amino acid.

Once administered intravenously to patients, the formulations object of the present invention do not provoke ulcerative damages, nor thrombophlebitis, at the site of injection.

In the present description, with the term basic amino acid we preferably intend a basic α -amino acid; preferred basic amino acids are selected from the group consisting of arginine, histidine and lysine.

5 Even more preferably, the formulations object of the present invention comprise arginine.

With the term formulation comprising estramustine phosphate we intend any formulation of estramustine phosphate, as the active ingredient, either in the acid form or as a
10 pharmaceutically acceptable salt for parenteral use such as, for instance, the salts of estramustine phosphate with basic amino acids themselves or with N-methyl glucamine, otherwise referred to as meglumine.

Preferably, the formulations of the invention comprise
15 estramustine phosphate in the form of its arginine or meglumine salt.

From the foregoing, it is clear to the man skilled in the art that the formulations of the present invention may
20 contain the basic amino acid, either in the form of its salt with estramustine phosphate as well as in admixture with an estramustine phosphate salt for parenteral use.

According to a preferred embodiment of the invention, it is
25 herewith provided a formulation for parenteral use comprising estramustine phosphate, in the form of its arginine salt, optionally in admixture with additional amounts of a basic amino acid, e.g. arginine.

30 Another preferred embodiment of the invention is a formulation for parenteral use comprising estramustine phosphate, in the form of its meglumine salt, in admixture with arginine.

35 In another preferred embodiment, estramustine phosphate is provided in lyophilised form and the basic amino acid is

provided in physiological solution. Formulations of this type may typically be provided as a kit.

The formulations object of the present invention may
5 further contain additional excipients for parenteral use.
Among them are also those excipients known in the art to
reduce the side effects associated with the parenteral
administration of cytotoxic agents (herewith referred to as
protective agents) such as, for instance, human albumin,
10 cyclodextrins, sulfoalkyl ether cyclodextrins and
derivatives thereof.

Preferably, the formulations of the invention additionally
comprise human albumin.

15 Very interestingly, the formulations of the invention also
containing a given amount of human albumin are unexpectedly
less toxic than the corresponding estramustine phosphate
formulations containing the same amount of human albumin,
as the sole protective agent against ulcerative damages and
20 thrombophlebitis at the site of injection.

It is therefore a further object of the invention a
formulation for parenteral use comprising estramustine
phosphate and a basic amino acid in admixture with human
25 albumin.

All of the above formulations are preferably intended for
intravenous use. As such, they can be administered to
patients either as a slow injection, e.g. over about 30
30 minutes to about 3 hours, or as a bolus injection, also
referred to as IV (intravenous) push.

In addition, the formulations of the invention provide a
very advantageous method for delivering estramustine
35 phosphate intravenously, even when high doses of the active
are needed.

It is therefore a further object of the invention a formulation for parenteral use comprising estramustine phosphate, as a single infusion dosage of the active exceeding 1300 mg, and a basic amino acid.

- 5 According to another preferred embodiment of the invention, it is further provided a formulation for parenteral use comprising estramustine phosphate, as a single infusion dosage of the active exceeding 950 mg/m², and a basic amino acid.

10

The formulations of the invention allow the administration of the active either as a single agent or, alternatively, according to a combined chemotherapy regimen.

- As an example, the above formulations can be administered
15 in combination with one or more chemotherapeutic agents such as, for instance, taxane derivatives, e.g. paclitaxel and docetaxel; camptothecin and derivatives thereof, e.g. CPT-11, 9-amino-camptothecin; anthracycline derivatives, e.g. doxorubicin, epirubicin, idarubicin, daunorubicin,
20 alkylcycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); etoposide; navelbine; vinblastine; platinum derivatives, e.g. carboplatin and cisplatin; angiogenesis inhibitors, e.g. Sugen SU-5416 and Sugen SU-6668; and the like,
25 optionally within liposomal formulations thereof.

- Therefore, it is a further object of the present invention a product containing a formulation for parenteral use comprising estramustine phosphate and a basic amino acid,
30 preferably arginine, and one or more chemotherapeutic agents, as a combined preparation for simultaneous, separate or sequential use in anticancer therapy.

Toxicology

- 35 To study the local irritant effects of estramustine phosphate (hereinafter referred to as EMP) after repeated intravenous administrations to rats, the active was

prepared, according to the present invention, either in the form of a salt with a basic amino acid and/or in admixture with a basic amino acid. Subsequently, the said preparations were dissolved in different vehicles such as water solution for injection and water solution for injection further containing human albumin (hereinafter referred to as HA). In addition, formulations of estramustine phosphate in the form of meglumine (hereinafter referred to as MEG) salts, optionally in admixture with HA only, were used for comparison. In particular, the following water for injection solutions (Table I) wherein either the active EMP as well as the basic amino acid or HA are expressed in terms of molar or weight ratios, were prepared and tested:

Table I

Solution	Molar ratio (mol/mol)	Weight ratio (w/w)
a)negative control water for inject.	-	-
b)positive control EMP-MEG salt	EMP:MEG=1:1	-
c)comparison EMP-MEG salt + HA	EMP:MEG=1:1	EMP:HA=1:0.21
d) EMP-Arg salt	EMP:Arg=1:1	
e) EMP-Arg salt + Arg	EMP:Arg=1:2	
f) EMP-Arg salt + HA	EMP:Arg=1:1	EMP:HA=1:0.21
g) EMP-MEG salt + Arg	EMP:MEG:Arg=1:1:2	

Male Sprague-Dawley rats were used because of their acceptance as a predictor of toxic change in man. The rats were 6 weeks old at the start of the study.

The above formulations were administered to groups of rats as a repeated intravenous injection during 3 days. Rats were then sacrificed: a half of the rats at the fourth day and a half at the fifth day.

- 5 The dose level of estramustine phosphate, in all the different tested solutions, was of 150 mg/kg/day. Clinical observations were recorded daily. Thrombophlebitic side effects resulted in a dark bluish/blackish coloration of the tail during the treatment period.
- 10 A score system based on tail coloration and its extension was used to evaluate the different tested formulations. The score system considered the formulation of estramustine phosphate salt with meglumine, solution (b), as the positive control (i.e. marked toxicity). Water for
- 15 injection (a) was administered to the control group as negative control (i.e. no toxicity signs). Histological evaluation was carried out on the tail of the rats treated with the composition of the invention. Estramustine phosphate in a water solution (b) induced, at
- 20 the used dose, local irritant effects at the injection site after the first administration and marked toxicity signs at the end of the experiment. Likewise, toxicity signs at the injection site were also observed for the comparison (c) EMP solution containing
- 25 human albumin, as the sole protective excipient against ulcerative damages at the injection site. On the contrary, a markedly reduced toxicity or even no toxicity was observed with the formulations of the invention containing arginine (d), (e) and (g), optionally
- 30 in admixture with human albumin (f). The histological evaluation of the tail of the rats treated with these formulations confirmed the above findings. In addition, the protective effect exerted by the basic amino acid, according to the invention, can clearly be
- 35 evidenced by considering the formulation (f) in comparison to the formulations (c) for which toxicity signs were observed. Very interestingly, in fact, both formulations

contained the same amount of human albumin with respect to the active.

- 5 It was thus concluded that estramustine phosphate in a water solution containing a basic amino acid, either in the form of a salt with estramustine phosphate or in admixture with the same, induced markedly less local irritant effects when compared with a water solution of estramustine phosphate itself.
- 10 Even more surprisingly, the formulations of the invention produced less local irritant effects also in comparison to analogous solutions of estramustine phosphate containing known protective excipients, for instance human albumin.
- 15 One particularly preferred schedule for administering the formulation of estramustine phosphate according to the invention is a single infusion given once weekly to a maximal dose of 4000 mg or 3500 mg/m². Another preferred schedule is the administration of a
- 20 single drug infusion once every two to four weeks. One schedule may be preferred over another in consideration of schedules with other optional concomitant therapy. These schedules may repeat in serial or as repetitive fashion.
- 25 The formulations of the present invention are useful in antitumor therapy, particularly in the treatment of prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer and cancers of the brain.
- 30 In addition to the above, we also noticed that arginine and the pharmaceutically acceptable salts thereof for parenteral use, exerted their protective effects against the occurrence of thrombophlebitis also when used in
- 35 combination with several other antineoplastic agents known to cause, or potentially cause, ulcerative damages at the site of injection, when administered intravenously.

It is therefore a further object of the invention a pharmaceutical formulation which comprises a parenterally acceptable carrier or diluent, an antineoplastic agent known to cause ulcerative damages at the site of injection upon intravenous administration, and arginine or a pharmaceutically acceptable salt thereof.

Besides estramustine phosphate, other antineoplastic agents are known to cause, at least potentially, ulcerative damages and thrombophlebitis at the site of injection. Preferably, these antineoplastic agents according to the invention are selected from the group consisting of anthracycline derivatives such as doxorubicin, epirubicin, idarubicin, daunorubicin and alkycycline (4-demethoxy-3'-deamino-3'-aziridiny-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); and angiogenesis inhibitors such as Sugen SU-5416 and Sugen SU-6668.

From the foregoing, it is a further object of the invention the use of arginine, or pharmaceutically acceptable salts thereof, in the preparation of a medicament for the treatment and prevention of side-effects associated with the intravenous administration of antineoplastic agents.

As set forth above, these side-effects comprise ulcerative lesions and thrombophlebitis at the site of injection.

In the present description, unless otherwise specified, with the term pharmaceutically acceptable salt of arginine, we intend any salt for parenteral use including, preferably, the arginine addition salt with hydrochloric, glutamic or aspartic acid.

The formulations object of the present invention are prepared according to conventional techniques adopted in the preparation of pharmaceutical forms for parenteral use.

25
22

17

Typically, a proper amount of estramustine phosphate is dispersed in water and then dissolved by adding at least an equimolar amount of a basic amino acid, for instance arginine.

- 5 A further amount of the given amino acid, e.g. arginine, can be present in order to reach an estramustine phosphate:arginine molar ratio higher than 1:1, respectively.

- 10 Alternatively, a proper amount of estramustine phosphate in the form of a pharmaceutically acceptable salt for parenteral use, e.g. estramustine phosphate meglumine salt, either as a dry powder or into a lyophilised form, is dissolved in a pharmaceutically acceptable solution for parenteral use, for instance sterile water or aqueous
- 15 dextrose solution, e.g. 5% dextrose in water for intravenous administration, and then admixed with a proper amount of a basic amino acid, for instance arginine.

- The above admixture is then stirred, sterilised, and subsequently lyophilised according to conventional
- 20 techniques.

- The freeze-dried formulation is prepared and stored in vials for injection; the addition of a proper amount of sterile water or a physiological solution for parenteral use enables the preparation of the final formulation to be
- 25 injected.

- The above method is also suitable for preparing high dosages estramustine phosphate formulations whilst maintaining the desired weight or molar ratio between the
- 30 components.

- The unit strength of the formulation to be injected depended on the concentration of the active in the solution itself and, of course, on the filling volume of the vials used to prepare the final formulation.

- 35 Additionally, besides the aforementioned human albumin, cyclodextrins, sulfoalkyl ether cyclodextrins and

derivatives thereof, the formulations of the invention may optionally contain additional pharmaceutically acceptable excipients for parenteral administration such as, for instance, bulking agents, e.g. lactose or mannitol, pH buffering agents, anti-oxidant agents, preservative agents, tonicity adjusters and the like.

From the foregoing, it is clear to the man skilled in the art that the above methods for preparing the estramustine formulations apply as well to arginine-containing formulations comprising antineoplastic agents other than estramustine phosphate, and known to cause, or potentially cause, thrombophlebitic side-effects upon intravenous administration.

The following examples are herewith intended to better illustrate the present invention without representing any limitation to it.

Example 1

Preparation of estramustine phosphate N-methyl glucamine salt in admixture with human albumin (estramustine phosphate:meglumine=1:1 molar ratio, estramustine phosphate:albumin=1:0.21 weight ratio)

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 120.8 mg of N-methyl-glucamine were then added under stirring to the watery dispersion of the active and, after a few minutes, a clear solution was obtained. 0.250 ml of a commercially available solution of human albumin at 25% concentration were added whilst maintaining the solution under stirring.

The obtained solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 6.25 mg/ml of human albumin (1:0.21 weight ratio respectively).

A

A solution prepared as previously described, properly sterilised by filtration, was tested for its local vein tolerability in rats.

5

Example 2

The formulation described in Example 1 was also prepared by dissolving the commercially available Estracyt® freeze-dried formulation containing 300 mg/vial of the active. The reconstitution of the formulation was made by using 10 ml
10 of a 6.25 mg/ml human albumin solution so as to obtain a final concentration of 30 mg/ml of estramustine phosphate and 6.25 mg/ml of human albumin (1:0.21 weight ratio respectively).

The albumin solution could be prepared either by dissolving
15 in water a proper amount of human albumin as a dry powder or by properly diluting a commercially available human albumin solution.

Example 3

20 **Preparation of estramustine phosphate arginine salt (estramustine phosphate:arginine=1:1 molar ratio)**

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 101 mg of arginine base were then added to the
25 watery dispersion of the active whilst maintaining under stirring until a clear solution was obtained.

The prepared solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate and 10.1 mg/ml of
30 arginine (1:1 molar ratio respectively).

A solution prepared as previously described, properly sterilised by filtration, was tested for its local vein tolerability in rats.

Example 4

Preparation of estramustine phosphate arginine salt in admixture with arginine (estramustine phosphate:arginine=1:2, molar ratio)

5 300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 202 mg of arginine base were then added to the watery dispersion of the active whilst maintaining under stirring until a clear solution was obtained. The basic pH
10 of the obtained solution was brought to the physiological value of about 7.5 by slow addition of diluted hydrochloric acid.

The solution was then brought to the final volume of 10 ml with water so as to reach a final concentration of 30 mg/ml
15 of estramustine phosphate and 20.2 mg/ml of arginine (1:2 molar ratio respectively).

A solution prepared as previously described, properly sterilised by filtration, was tested for its local vein tolerability in rats.

20

Example 5

**Preparation of estramustine phosphate arginine salt in admixture with human albumin (estramustine phosphate:arginine=1:1 molar ratio, estramustine
25 phosphate:albumin=1:0.21 weight ratio)**

300 mg of estramustine phosphate were weighed in a beaker and dispersed by means of magnetic stirring in 5 ml of water. 101 mg of arginine base were then added to the watery dispersion of the active whilst maintaining under
30 stirring until a clear solution was obtained. 0.250 ml of a commercially available solution of human albumin at 25% concentration were added, maintaining the solution under stirring.

The solution was then brought to the final volume of 10 ml
35 with water so as to reach a final concentration of 30 mg/ml of estramustine phosphate, 10.1 mg/ml of arginine and 6.25 mg/ml of human albumin. The molar and weight ratios between

21

the components of the solution were as follows:
estramustine phosphate:arginine 1:1 molar ratio,
estramustine phosphate:human albumin 1:0.21 weight ratio.
A solution prepared as previously described, properly
5 sterilised by filtration, was tested for its local vein
tolerability in rats.

Example 6

Preparation of estramustine phosphate N-methyl glucamine
10 salt in admixture with arginine (estramustine
phosphate:meglumine:arginine=1:1:2 molar ratio)

300 mg of estramustine phosphate were weighed in a beaker
and dispersed by means of magnetic stirring in 5 ml of
water. 120.8 mg of N-methyl-glucamine were then added under
15 stirring to the watery dispersion of the active and, after
a few minutes, a clear solution was obtained. A total
amount of 202 mg of arginine was then added to the above
described solution by using a suitable mixture of arginine
base and hydrochloride, in order to maintain the pH around
20 the physiological value of 7.5.

The solution was then brought to the final volume of 10 ml
with water so as to reach a final concentration of 30 mg/ml
of estramustine phosphate and 20.2 mg/ml of arginine (1:2
molar ratio respectively).

25 A solution prepared as previously described, properly
sterilised by filtration, was tested for its local vein
tolerability in rats.

Example 7

30 The formulation described in Example 6 was also prepared by
solubilization of the commercially available Estracyt®
freeze-dried formulation containing 300 mg/vial of the
active. The reconstitution of the formulation was made by
using 10 ml of a 20.2 mg/ml arginine solution so as to
35 reach a final concentration of 30 mg/ml of estramustine
phosphate and 20.2 mg/ml of arginine (1:2 molar ratio
respectively).

The arginine solution used to dissolve the freeze-dried formulation contained a proper mixture of arginine base and hydrochloride, in order to maintain the pH around the physiological value of 7.5.

5

Example 8

Preparation of a formulation comprising doxorubicin and arginine in a 1:1 molar ratio.

Doxorubicin hydrochloride (40 mg) and arginine (12 mg) were weighed in a flask and the admixture was dissolved in a physiological solution (15 ml) of NaCl 0.9% w/v, under stirring. A solution of hydrochloric acid was then added up to pH = 3. The solution thus prepared was then diluted with the above physiological solution up to a final volume of 20 ml so as to reach a final concentration of 2 mg/ml of doxorubicin and 0.6 mg/ml of arginine (molar ratio 1:1, respectively).

Example 9

Preparation of a formulation comprising doxorubicin and arginine in a 1:2 molar ratio

By working as above described in example 8 and by using an amount of arginine as twice, that is 24 mg of arginine per 40 mg of doxorubicin hydrochloride, a solution comprising doxorubicin and arginine in a molar ratio 1:2, respectively, was thus prepared.

Example 10

Preparation of a formulation comprising Sugen SU-5416 and arginine in a 1:1 molar ratio

10 ml of an aqueous solution of NaCl (0.9% w/v) were diluted with 10 ml of water in a flask so as to obtain a solution of NaCl at 0.45% w/v.

Arginine hydrochloride (39.79 mg) was then added to the resultant solution and dissolved by shaking.

The solution thus prepared was used to dilute a solution of Sugen SU-5416 having the following composition:

32
29

Component	Amount % (w/v) in the formulation
Sugen SU 5416	0.45 %
PEG 400	45 %
Benzyl alcohol	2 %
Cremophor EL	31.5 %
Anhydrous Ethanol	Up to 100

The dilution was carried out by admixing one part of the formulation containing the active Sugén SU-5416 with two parts of the solution containing arginine, so as to obtain a final solution of Sugén SU-5416 and arginine in a molar ratio 1:1.

Example 11

- 10 **Preparation of a formulation comprising Sugén SU-5416 and arginine in a 1:2 molar ratio**
- By working as above described in example 10 and by using an amount of arginine hydrochloride as twice, that is 79.58 mg, a solution comprising Sugén SU-5416 and arginine in a 15 1:2 molar ratio, respectively, was thus prepared.

CLAIMS

1. A pharmaceutical formulation which comprises a parenterally acceptable carrier or diluent and estramustine phosphate and a basic amino acid.
5
2. A formulation according to claim 1 wherein the basic amino acid is arginine.
- 10 3. A formulation according to claim 1 or 2 wherein estramustine phosphate is in the form of a pharmaceutically acceptable salt for parenteral use.
- 15 4. A formulation according to claim 3 wherein the estramustine phosphate is in the form of a salt with arginine, histidine, lysine or N-methyl glucamine.
- 20 5. A formulation according to claim 4 wherein the estramustine phosphate is in the form of a salt with arginine or N-methyl glucamine.
- 25 6. A formulation according to anyone of the preceding claims comprising estramustine phosphate and arginine in a molar ratio lower than 1:1.
7. A formulation according to claim 6 comprising estramustine phosphate and arginine in a molar ratio of about 1:2.
- 30 8. A formulation according to claim 1 which further comprises human albumin, a cyclodextrin or a sulfoalkyl ether cyclodextrin.
- 35 9. A formulation according to claim 1 which further comprises human albumin.

10. A formulation according to any one of the preceding claims which is in single infusion dosage form comprising at least 1300 mg of the estramustine phosphate.
- 5 11. A formulation according to any one of the preceding claims which is in single infusion dosage form comprising at least 950 mg/m² of the estramustine phosphate.
12. A formulation according to claim 10 or 11 wherein the
10 basic amino acid is arginine.
13. A formulation according to any one of the preceding claims for intravenous use.
- 15 14. A formulation according to any one of the preceding claims for use in the treatment of cancer.
15. A formulation as claimed in claim 14 wherein the cancer is prostate cancer, breast cancer, melanoma, lung
20 cancer, pancreatic cancer, colorectal cancer, ovarian cancer or cancer of the brain.
16. A formulation according to claim 1 wherein the parenterally acceptable carrier is a physiological solution
25 for parenteral use which contains the basic amino acid, and the estramustine phosphate is in lyophilised form.
17. A product which comprises:
(i) a pharmaceutical formulation which comprises a
30 parenterally acceptable carrier or diluent and estramustine phosphate and a basic amino acid, and
(ii) one or more chemotherapeutic agents,
as a combined preparation for simultaneous, separate or sequential use in anticancer therapy.
- 35 18. A product according to claim 17 wherein the basic amino acid is arginine.

19. A product according to claim 17 or 18 wherein the chemotherapeutic agent is selected from the group consisting of taxane derivatives such as paclitaxel and docetaxel; camptothecin and derivatives thereof such as
5 CPT-11 and 9-amino-camptothecin; anthracycline derivatives such as doxorubicin, epirubicin, idarubicin, daunorubicin, alkylcycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); etoposide; navelbine; vinblastine; platinum derivatives
10 such as carboplatin and cisplatin; angiogenesis inhibitors such as Sugen SU-5416 and Sugen SU-6668; optionally within liposomal formulations thereof.
20. A product according to claim 17 for intravenous use.
15
21. A product according to claim 17 for use in the treatment of prostate cancer, breast cancer, melanoma, lung cancer, pancreatic cancer, colorectal cancer, ovarian cancer or cancer of the brain.
20
22. A formulation as defined in claim 13 for use in suppressing or reducing the side-effects associated with the intravenous administration of estramustine phosphate and pharmaceutically acceptable salts thereof.
25
23. A formulation according to claim 22 wherein the side effects comprise ulcerative lesions and thrombophlebitis at the site of injection.
- 30 24. A product which comprises estramustine phosphate in lyophilised form and a physiological solution for parenteral use containing a basic amino acid.
- 35 25. Use, in the manufacture of a medicament for parenteral administration, of estramustine phosphate and a basic amino acid.

26. Use according to claim 25 wherein the medicament is for intravenous administration.

27. A pharmaceutical formulation which comprises a parenterally acceptable carrier or diluent, an antineoplastic agent known to cause ulcerative damages at the site of injection upon intravenous administration, and arginine or a pharmaceutically acceptable salt thereof.

28. A formulation according to claim 27 wherein the antineoplastic agent is selected from the group consisting of anthracycline derivatives such as doxorubicin, epirubicin, idarubicin, daunorubicin and alkycycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); and angiogenesis inhibitors such as Sugen SU-5416 and Sugen SU-6668.

29. Use of arginine, or of pharmaceutically acceptable salts thereof, in the preparation of a medicament for the treatment and prevention of side-effects associated with the intravenous administration of antineoplastic agents.

30. Use according to claim 29 wherein the side-effects comprise ulcerative lesions and thrombophlebitis at the site of injection.

31. Use according to claim 29 wherein the antineoplastic agent is selected from the group consisting of estramustine phosphate; anthracycline derivatives such as doxorubicin, epirubicin, idarubicin, daunorubicin and alkycycline (4-demethoxy-3'-deamino-3'-aziridinyl-4'-methylsulfonyl-daunorubicin; internal code PNU 159548); and angiogenesis inhibitors such as Sugen SU-5416 and Sugen SU-6668.

37
34

INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/EP 00/08983

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K31/565 A61K31/66 A61K9/08 A61P35/00 A61K47/18		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) WPI Data, PAJ, CHEM ABS Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 785 976 A (K.WESTESEN ET AL.) 28 July 1998 (1998-07-28) claims 1,4,5,8-11,14,15,17,18,20 column 15, line 15 - line 17 column 15, line 32 - line 33	1,8,9, 13,25,26
E	WO 00 59475 A (LIPOCINE INC.,U.S.A.) 12 October 2000 (2000-10-12) claims 1-4,8,19,48-50,57-59,61,63,65,67-78,85-90 claims 110,112-115 page 13, line 24 - line 27 -/-	1,8,13, 25,26
☒ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "A" document member of the same patent family		
Date of the actual completion of the international search 19 January 2001		Date of mailing of the international search report 29/01/2001
Name and mailing address of the ISA European Patent Office, P.B. 5518 Patendlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Scarponi, U

29

INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/EP 00/08983

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 807 572 A (S. KIM ET AL.) 15 September 1998 (1998-09-15) claims 1,3,25-39 column 5, line 39 - line 41 column 6, line 58 -column 7, line 3 column 7, line 41 column 7, line 60 - line 67 table 1	27-31
A	EP 0 612 530 A (SNOW BRAND MILK PRODUCTS CO. LTD.,JP) 31 August 1994 (1994-08-31) claims 1-4 table 4	27-31

39
36

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Appl. No.
PCT/EP 00/08983

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5785976 A	28-07-1998	CA 2091152 A US 5885486 A AU 676279 B AU 6225394 A EP 0687172 A FI 954143 A JP 8507515 T NO 953461 A NZ 262541 A WO 9420072 A	06-09-1994 23-03-1999 06-03-1997 26-09-1994 20-12-1995 19-10-1995 13-08-1996 06-11-1995 24-04-1997 15-09-1994
WO 0059475 A	12-10-2000	NONE	
US 5807572 A	15-09-1998	US 6071534 A US 5723147 A	06-06-2000 03-03-1998
EP 612530 A	31-08-1994	JP 6247872 A AT 197404 T AU 678421 B AU 5630794 A CA 2116192 A DE 69426244 D US 5510327 A ZA 9401198 A	06-09-1994 11-11-2000 29-05-1997 01-09-1994 24-08-1994 14-12-2000 23-04-1996 20-09-1994



US005780446A

United States Patent [19]

Ramu

[11] Patent Number: 5,780,446

[45] Date of Patent: Jul. 14, 1998

[54] FORMULATIONS OF VESICANT DRUGS AND METHODS OF USE THEREOF

[75] Inventor: Avner Ramu, Houston, Tex.

[73] Assignee: Baylor College of Medicine, Houston, Tex.

[21] Appl. No.: 700,742

[22] Filed: Jul. 9, 1996

[51] Int. Cl.⁶ A61K 31/70; A61K 31/525

[52] U.S. Cl. 514/34; 514/251

[58] Field of Search 514/34, 251

[56] References Cited

U.S. PATENT DOCUMENTS

4,124,598	11/1978	Hearst et al.	260/343.21
4,169,204	9/1979	Hearst et al.	546/270
4,196,281	4/1980	Hearst et al.	536/28
4,312,883	1/1982	Baccichetti et al.	424/279
4,398,031	8/1983	Bender et al.	549/282
4,827,016	5/1989	Morgan	560/16
4,906,100	3/1990	Rice et al.	356/417
5,047,245	9/1991	Bally et al.	424/450
5,049,392	9/1991	Weiner et al.	424/450
5,106,951	4/1992	Morgan, Jr. et al.	530/391.9
5,171,578	12/1992	Bally et al.	424/450
5,212,291	5/1993	Murdock et al.	536/6.4
5,216,176	6/1993	Heindel et al.	549/280
5,308,874	5/1994	Sanchez et al.	514/731
5,356,929	10/1994	Heindel et al.	514/435
5,461,080	10/1995	Sanchez et al.	514/731
5,468,499	11/1995	Chan et al.	424/450
5,475,092	12/1995	Chari et al.	530/391.7

OTHER PUBLICATIONS

Goodman & Gilman's *The Pharmacological Basis of Therapeutics*, 9th ed. (Ed. J.G. Hardman, et al.) (1996) "Chemotherapy of Neoplastic Diseases," MacGraw Hill, New York, pp. 1225-1237, 1242-1243, 1257-1260, 1263-1266.

Rudolph et al. (1976) "Skin Ulcers Due to Adriamycin." *Cancer* 38:1087-1094.

Devita et al., *Cancer: Principles and Practice of Oncology*, 3rd edition, Philadelphia, Lippincott, 1989:2386.

Dorr (1990) "Antidotes to Vesicant Chemotherapy Extravasations." *Blood Rev.* 4:41-60.

Andersson and Dahlstrom (1993) "Clinical Results After Doxorubicin Extravasation Treated With Excision Guided by Fluorescence Microscopy." *Eur. J. Cancer* 29A:1712-1714.

Lichtenstien and Goldman (1970) "Riboflavin-Methotrexate Interactions: Photochemical Reaction and Competition for Transport in the L1210 Mouse Leukemia Cell." *Biochem. Pharmacol.* 19:1229-1239.

Granzow et al. (1995) "Riboflavin-mediated Photosensitization of Vinca Alkaloids Distorts Drug Sensitivity Assays." *Cancer Res.* 55:4837-4843.

Yajima and Mizunoya (1981) "Kinetics of Photoinactivation and Photooxidation of Mitomycin C in the Presence of Riboflavin." *J. Biochem.* 89:929-936.

Sanfilippo et al. (1968) "Photodynamic Action of Daunomycin I—Effect on Bacteriophage T2 and Bacteria." *Giorn. Microbiol.* 16:50.

Verini et al. (1968) "Photodynamic Action of Daunomycin II—Effect on Animal Viruses." *Giorn. Microbiol.* 16:55.

Di Marco et al. (1972) "Interaction of Daunomycin with Nucleic Acids: Effect of Photoirradiation of the Complex." *Experientia* 28:327-329.

Daugherty et al. (1979) "Direct In Vitro Photoaffinity Labeling of DNA with Daunorubicin, Adriamycin, and Rubidazole." *Biochem. Biophys. Acta* 565:13-21.

Williams and Tritton (1981) "Photoinactivation of Anthracyclines." *Photochem. Photobiol.* 34:131-134.

Gray and Phillips (1981) "Ultraviolet Photoirradiation of Daunomycin And DNA—Daunomycin Complexes." *Photochem. Photobiol.* 33:297-303.

Gray et al. (1982) "Photosensitized Degradation of DNA by Daunomycin." *Photochem. Photobiol.* 36:49-57.

Yee et al. (1984) "Photoaffinity Labeling of the Sarcoma 180 Cell Surface by Daunomycin." *Cancer Res.* 44:1898-1903.

Luedke et al. (1979) "Histopathogenesis of Skin and Subcutaneous Injury Induced by Adriamycin." *Plast. Reconstr. Surg.* 63:463-465.

Hearst (1989) "Photochemistry of the Psoralens." *Chemical Research in Toxicology*, 2:69-75.

Anderson and Vorhees (1980) "Psoralen Photochemotherapy of Cutaneous Disorders." *Ann. Rev. Pharmacol. Toxicol.* 20:235-257.

Kharasch and Novak (1981) "The Molecular Basis for Complexation of Adriamycin with Flavin Mononucleotide and Flavin Adenine Dinucleotide." *Arch. Biochem. Biophys.* 212:20-36.

Byrom and Turnbull (1967) "Excited States of Flavine Coenzymes—II. Anaerobic Oxidation of Amino Acids by Excited Riboflavin Derivatives." *Photochem. Photobiol.* 6:125-131.

Friedrich (1988) in *Vitamins*, Friedrich, ed., W. de Gruyter, Berlin, pp. 403-471.

Kim et al. (1993) "Photosensitized Formation of Ascorbate Radicals by Riboflavin: An ESR Study." *Photochem. Photobiol.* 57:777-784.

Chinami et al. (1984) "Semiquinone Formation of Adriamycin by Oxidation at para-OH Residue." *Biochem. Int.* 8:299-304.

Silva and Godoy (1994) "Riboflavin Sensitized Photooxidation of Tyrosine." *Int. J. Vit. Nutr. Res.* 64:253-256.

(List continued on next page.)

Primary Examiner—Kimberly Jordan
Attorney, Agent, or Firm—Medlen & Carroll, LLP

[57] ABSTRACT

The present invention relates to methods of treating, as well as preventing extravasation injury. In particular, the present invention pertains to photochemotherapeutic methods of prophylaxis and/or treatment of extravasation injury induced by vesicant antineoplastic drugs and other pharmaceutical formulations. In accordance with the present invention, extravasation injury is prevented or minimized by the coadministration of photoinactivation inducing compounds in a formulation comprising a vesicant antineoplastic or other pharmaceutical formulation, and subsequently exposing the injection or infusion site to photoexciting light.

41 Claims, 38 Drawing Sheets

OTHER PUBLICATIONS

- Brawley et al. (1993) "Hydrogen peroxide generation in a model paediatric parenteral amino acid solution." *Clin. Sci.* 85:709-712.
- Rosenfeld et al. (1984) "Fetal and Neonatal Medicine: Prevention of bronchopulmonary dysplasia by administration of bovine superoxide dismutase in preterm infants with respiratory distress syndrome." *J. Pediatr.* 105:781-785.
- Ramu et al. (1984) "Reversal of Acquired Resistance to Doxorubicin in P388 Murine Leukemia Cells by Perhexiline Maleate." *Cancer Res.* 44:144-148.
- Giorati (1983) "Yearly Review: Mechanisms of Photosensitization." *Photochem. Photobiol.* 38:745-751.
- Dorr et al. (1980) "Experimental Model of Doxorubicin Extravasation in the Mouse." *J. Pharmacol. Methods* 4:237-250.
- Dorr et al. (1980) "The Limited Role of Corticosteroids in Ameliorating Experimental Doxorubicin Skin Toxicity in the Mouse." *Cancer Chemother. Pharmacol.* 5:17-20.
- Averbuch et al. (1986) "Doxorubicin-Induced Skin Necrosis in the Swine Model: Protection With a Novel Radical Dimer." *J. Clin. Oncol.* 4:88-94.
- Dahlstrom et al. "Fluorescence Microscopic Demonstration and Demarcation of Doxorubicin Extravasation." *Cancer* 65:1722-1726 (1990).
- Iyengar. "Neural Differentiation As An Expression Of UV Sensitivity." *Acta. Anatomica* 143:236-240 (1981).
- Synderman and Krasna. "Adriamycin Extravasation Injuries." *Plastic & Reconstructive Surg.* 77:683-684 (1986).
- Braun-Falco et al. *Dermatology* (Springer-Verlag) New York. pp. 1189-1191 (1984).
- Dall'Acqua et al. "New Monofunctional Reagents for DNA as Possible Agents for the photochemotherapy of Psoriasis: Derivatives of 4,5'-Dimethylangelicin." *J. Med. Chem.* 24:178-184 (1981).
- Hida et al. "Effects Of Antioxidants On Adriamycin-Induced Microsomal Lipid Peroxidation." *Biol. Trace Element Res.* 47:111-116 (1995).
- Larson. "Alterations in Wound Healing Secondary to Infusion Injury." *Clinics in Plastic Surgery* 17(3):509-517 (1990).
- Lawrence et al. "Topical dimethylsulfoxide may prevent tissue damage from anthracycline extravasation." *Cancer Chemother Pharmacol* 23:316-318 (1989).
- Lee et al. Adriamycin Induced Myocardial Dysfunction In Vitro Is Mediated By Free Radicals. *Am. J. Physiol.* 261:H989-H9895 (1991).
- Loth and Eversmann. "Treatment Methods For Extravasations Of Chemotherapeutic Agents: A Comparative Study." *J. Hand Surg.* 11:388-396 (1986).
- Ludwig et al. "Prevention of Cytotoxic Drug Induced Skin Ulcers With DMSO and Alpha-Tocopherol." *Eur. J. Cu & Clin. Oncol.* 23:327-329 (1987).
- Okano et al. "Doxorubicin-Induced Skin Ulcer in the Piglet." *Cancer Treatment Reports* 67(12):1075-1078 (1983).
- Rudolph and Larson. "Etiology and Treatment of Chemotherapeutic Agent Extravasation Injuries: A Review." *Journal of Clinical Oncology.* 5(7):1116-1126 (1987).
- Soble et al. "Dose-dependent skin ulcers in mice treated with DNA binding antitumor antibiotics." *Cancer Chemother Pharmacol* 20:33-36 (1987).
- Speranza et al. "Cells Enriched for Catalase Are Sensitized to the Toxicities of Bleomycin, Adriamycin, and Paraquat." *J. Biol. Chem.* 268:19039-19043 (1993).
- Taylor et al. "Glutathione Peroxidase Protects Cultured Mammalian Cells From the Toxicity of Adriamycin and Paraquat." *Arch. Biochem. & Biophysics* 305:600-605 (1993).
- Thavaris et al. "Conservative Approach to the Treatment of Chemotherapy Induced Extravasation." *J. Derm. Surg. & Oncol.* 16:519-522 (1990).
- Wang et al. "Effect of Vitamin E Against Adriamycin-Induced Toxicity in Rabbits." *Ca. Res.* 40:1022-1027 (1980).
- Miura et al. "Generation of Adriamycin Radical by Interaction with Serum." *Res. Comm. Chem. Path. & Pharmacol.* 71:115-124 (1991).
- Ogura et al. "Riboflavin Deficiency Caused By Treatment with Adriamycin." *J. Nutritional Sci. & Vitaminology* 37:473-477 (1991).
- Pinto et al. "Adriamycin Induced Increase In Serum Aldosterone Levels: Effects in Riboflavin-Sufficient and Riboflavin-Deficient Rats." *Endocrinology* 127:1495-1501 (1990).
- Ralczyk et al. "Enhancement of Adriamycin-Induced Mortality during Riboflavin Administration and Riboflavin Deficiency in Rats." *Proceedings of the Society for Experimental Biology and Medicine* 188:495-499 (1988).
- Caffieri et al. "Photocycloaddition of 4,5'-Dimethylangelicin to Cytosine in the Photoreaction with DNA: Isolation of the Adduct." *Medicine Biologie* 11:387-391 (1983).
- Dall'Acqua et al. "Monofunctional 3,4' and 4,5'-Photocycloadducts Between 4,5'-Dimethylangelicin and Thymine." *Photochemistry and Photobiology* 37(4):373-379 (1983).
- Henst et al. "The reaction of the psoralens with deoxyribonucleic acid." *Quarterly Review of Biophysics* 17(1):1-44 (1984).

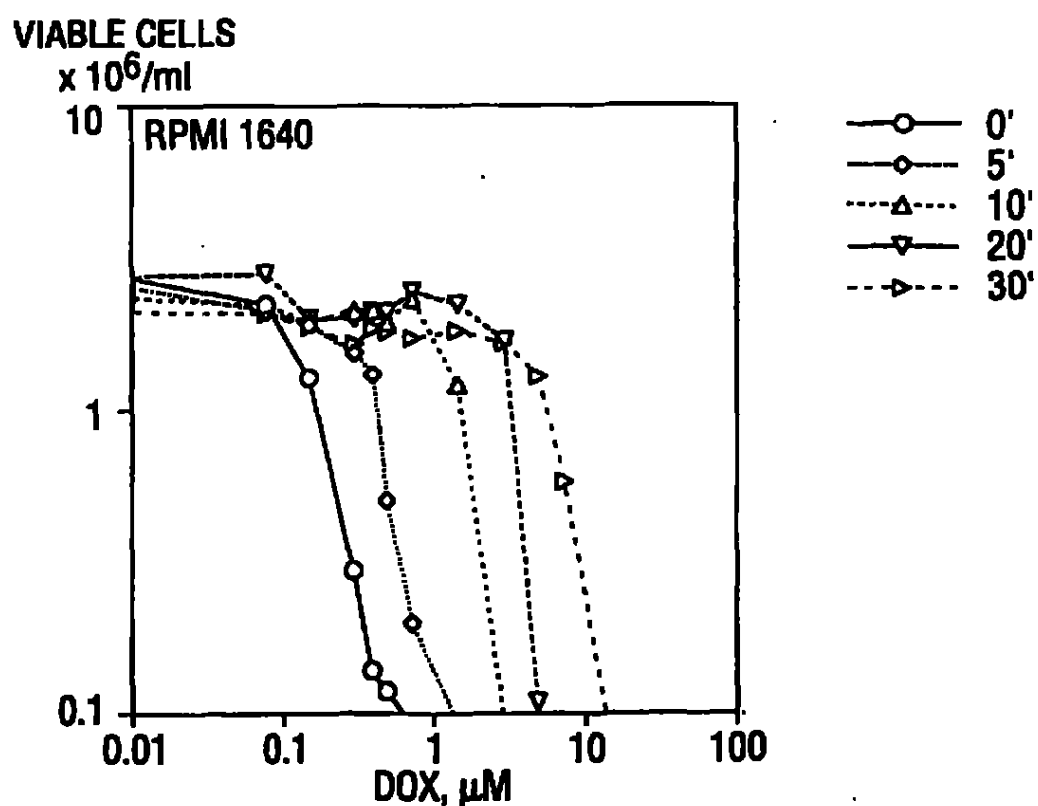


FIG. 1A

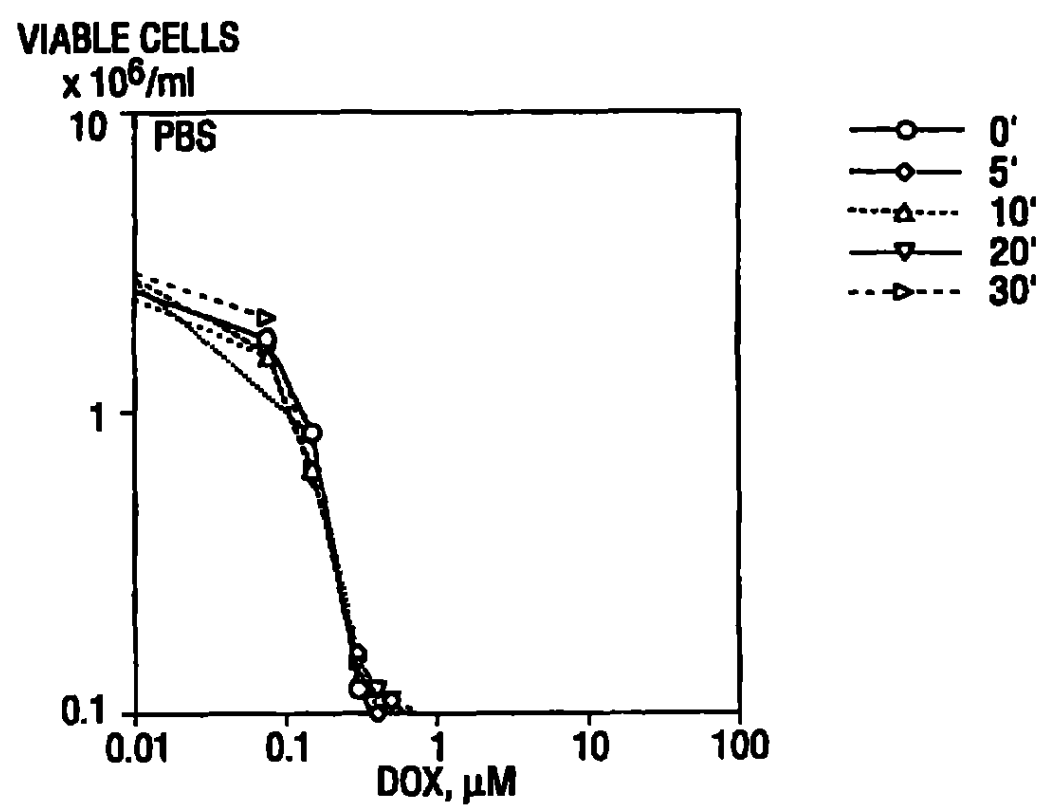


FIG. 1B

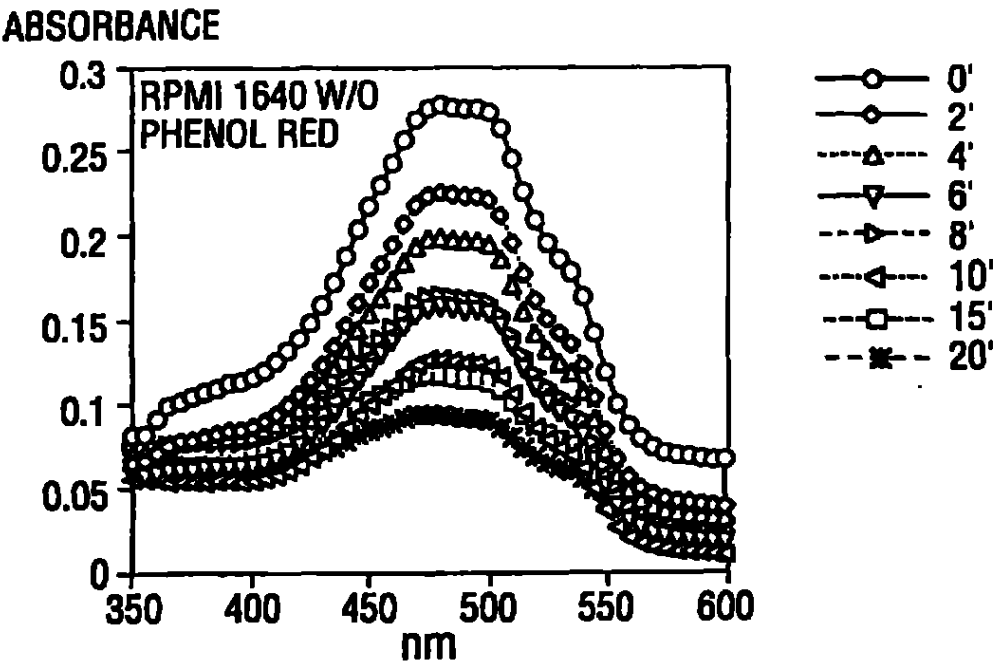


FIG. 2A

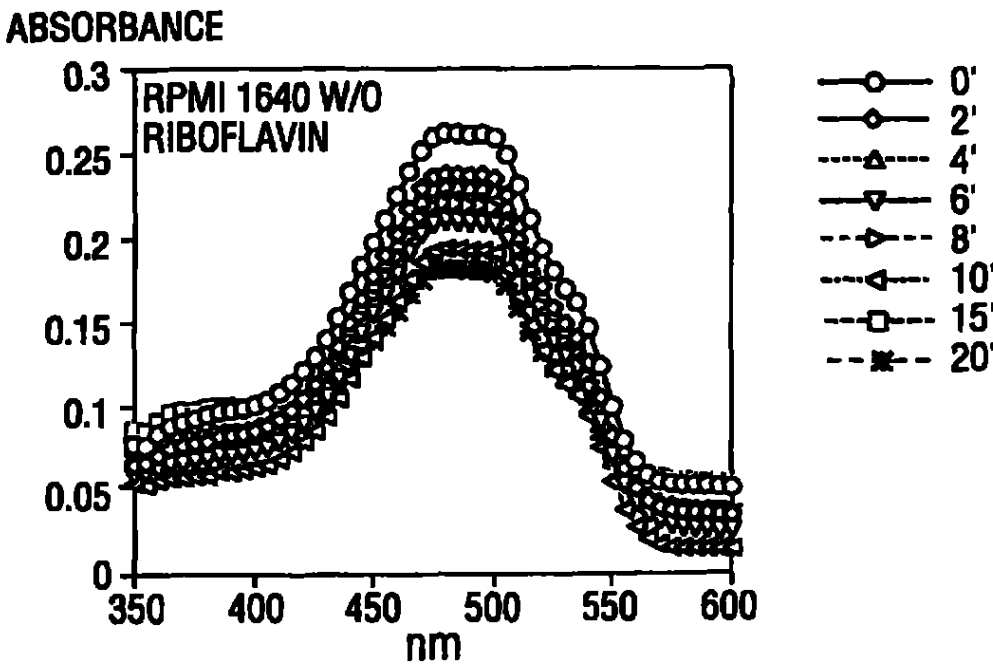


FIG. 2B

44
41

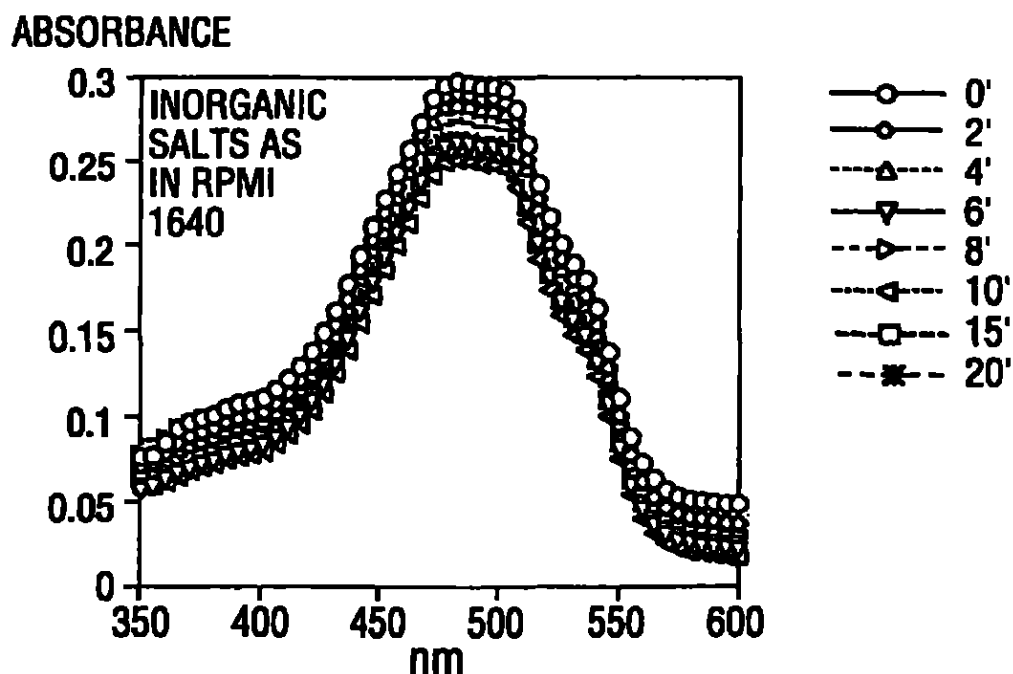


FIG. 2C

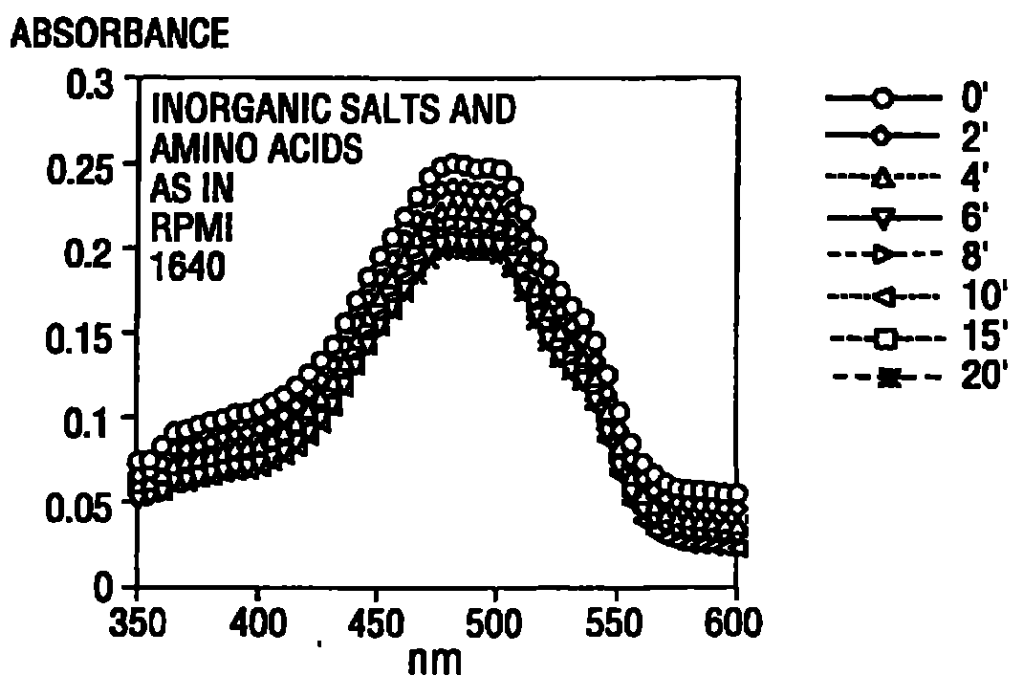


FIG. 2D

ABSORBANCE

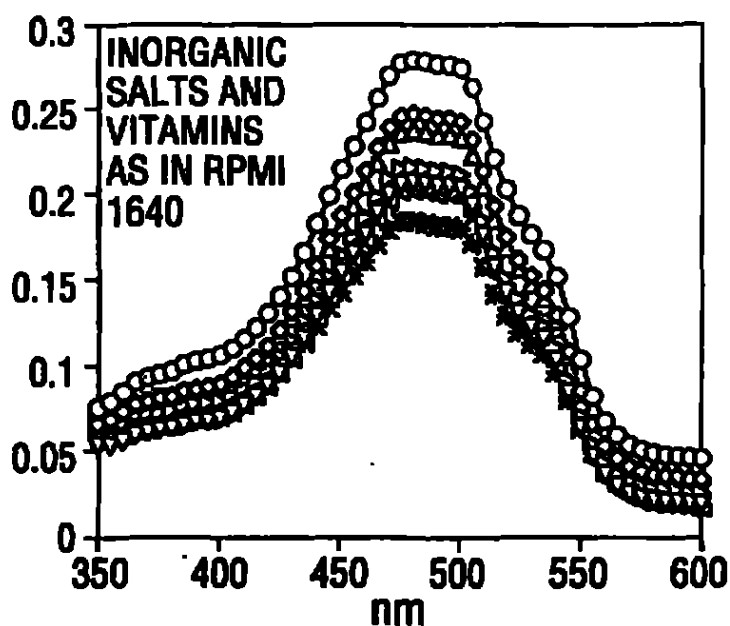


FIG. 2E

ABSORBANCE

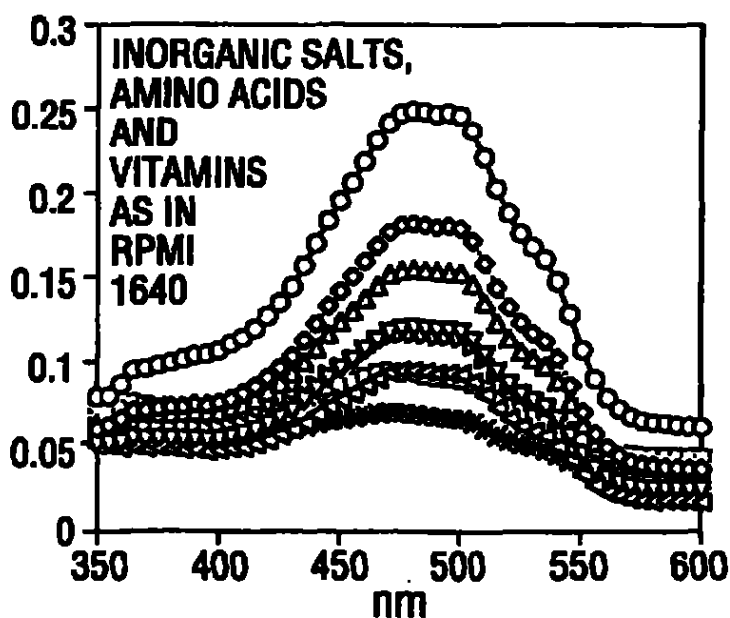


FIG. 2F

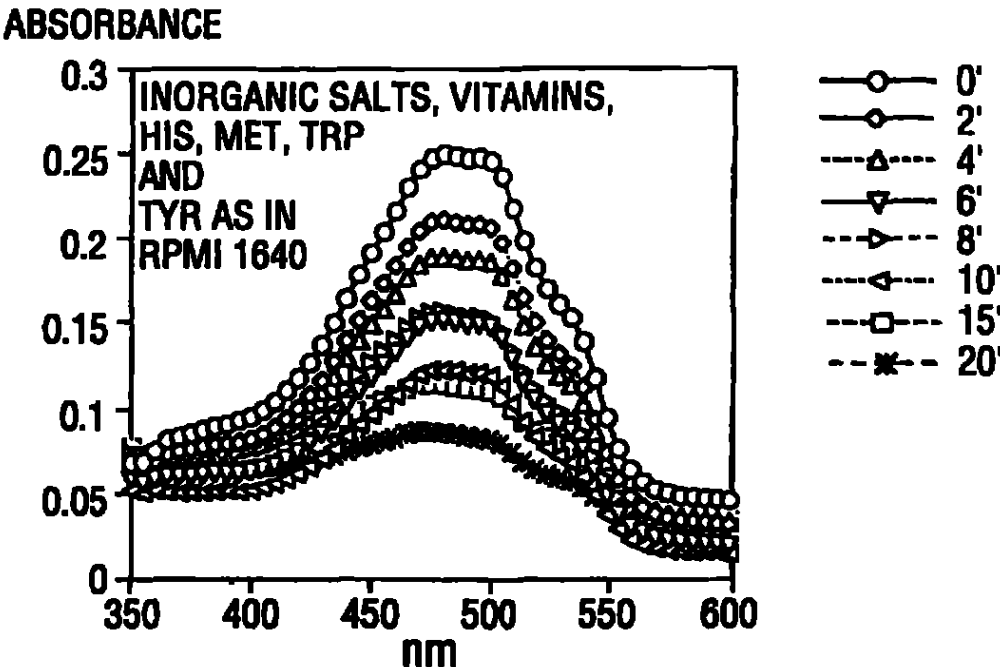


FIG. 3A

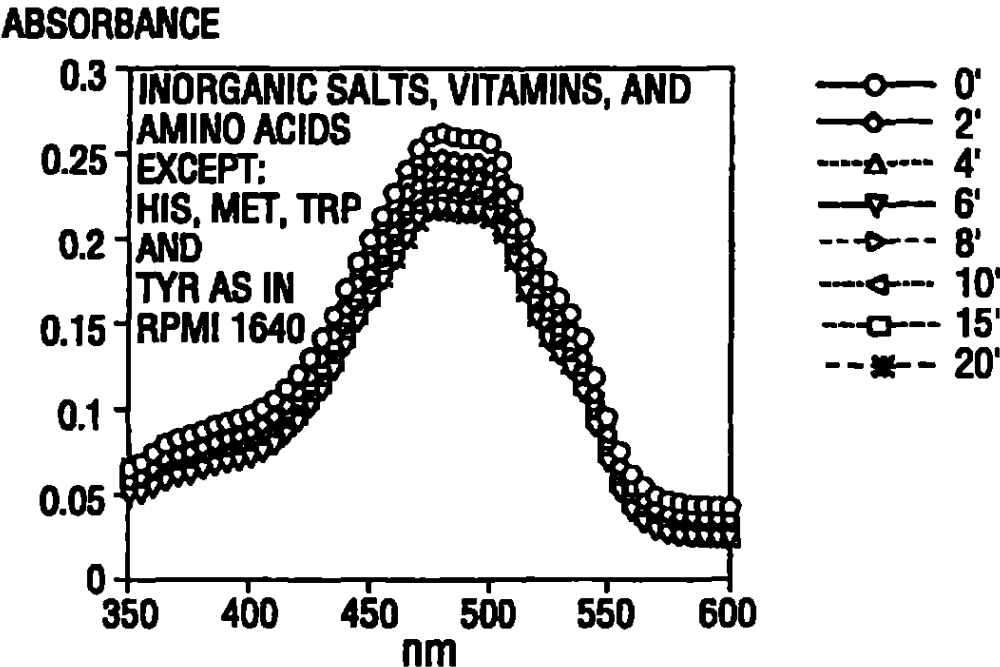


FIG. 3B

47
44

ABSORBANCE

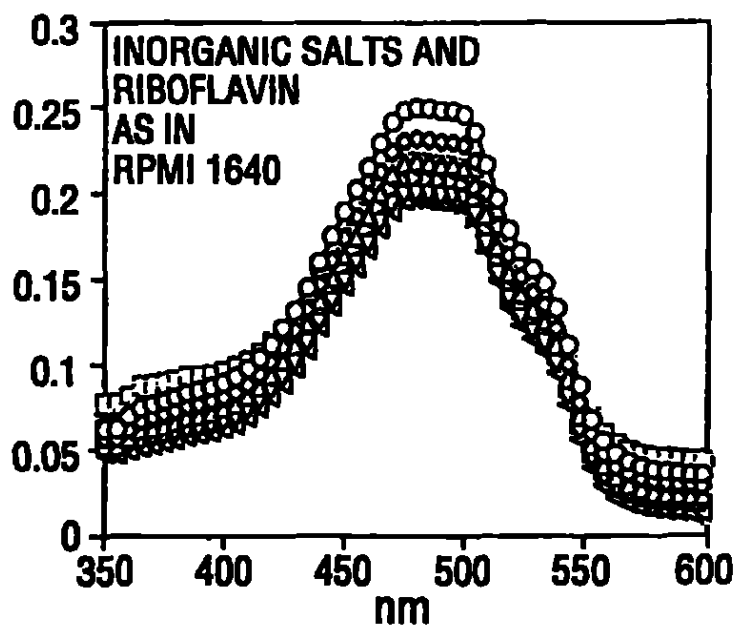


FIG. 3C

ABSORBANCE

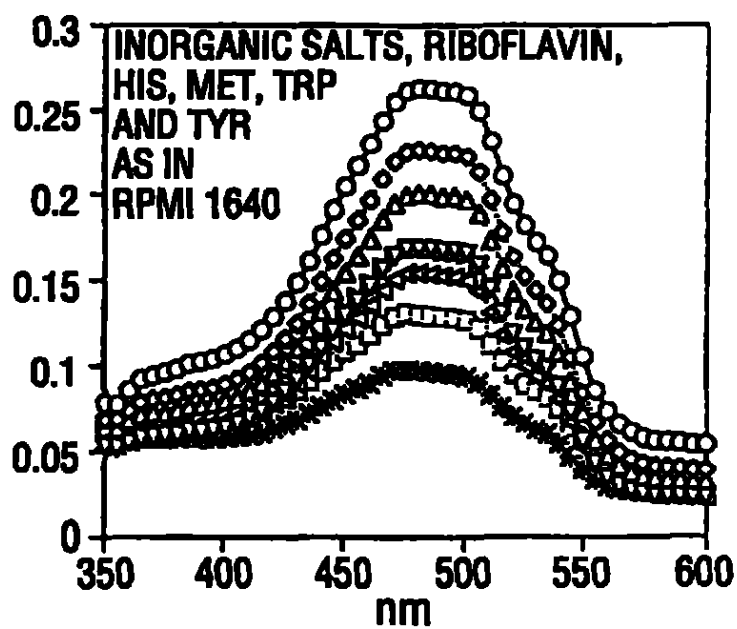
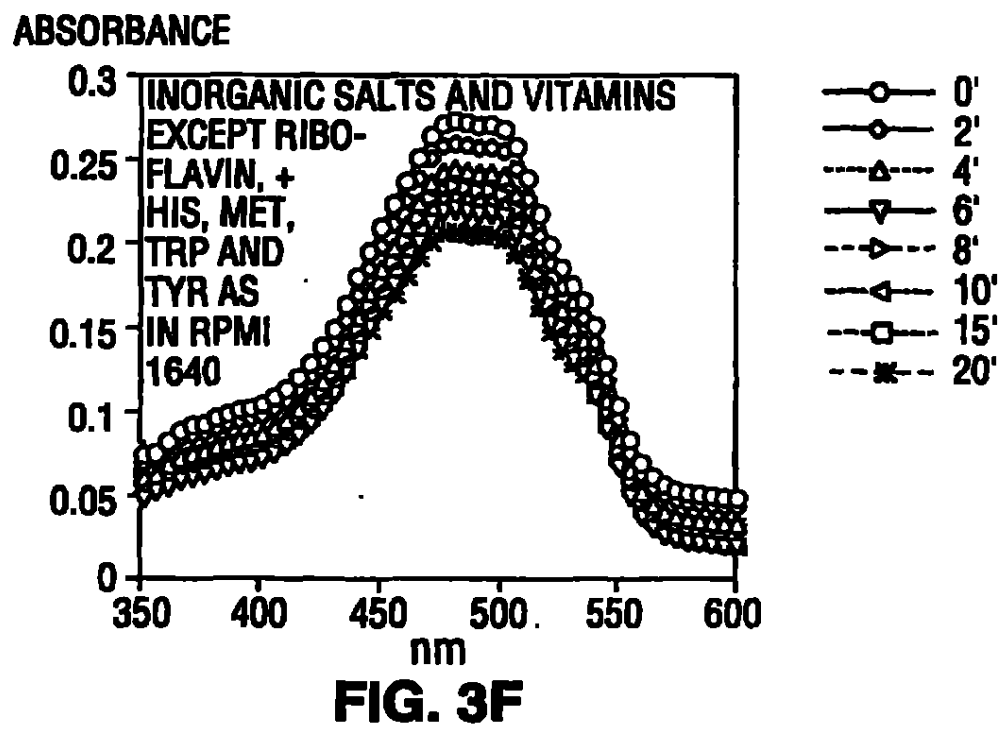
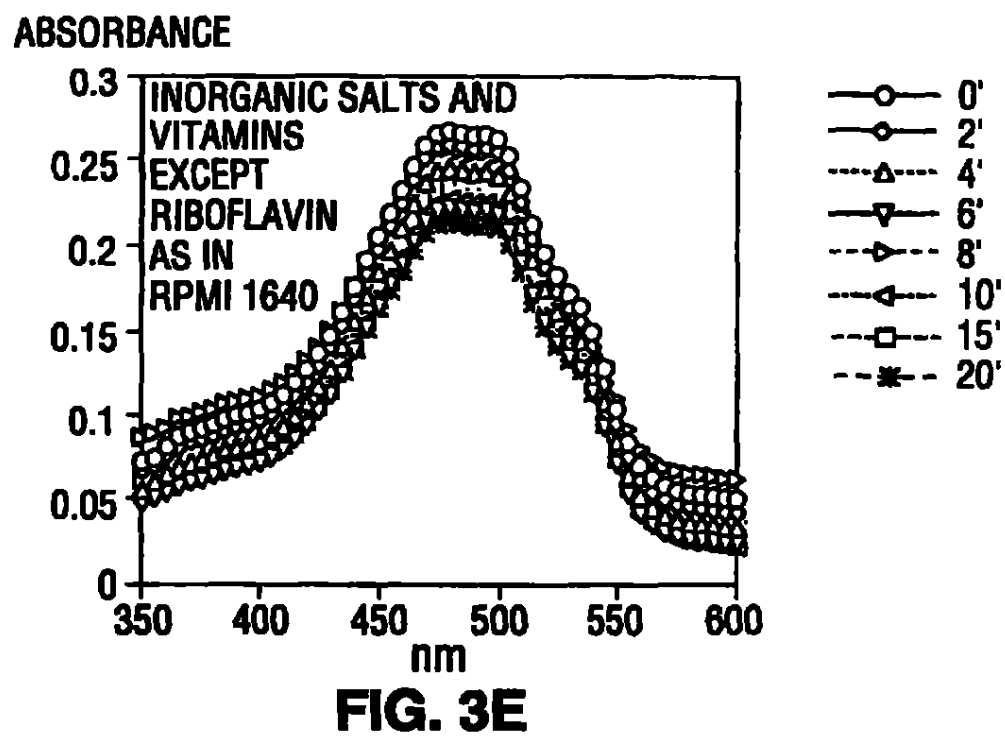


FIG. 3D



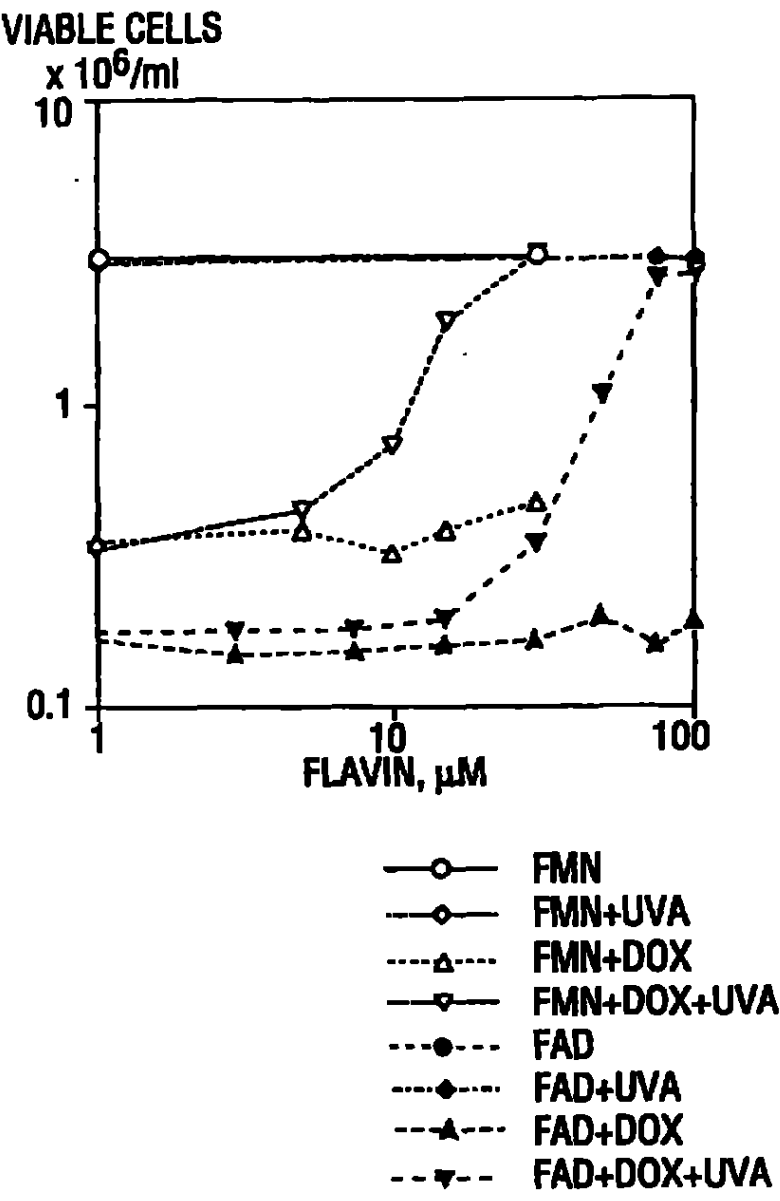


FIG. 4

50
47

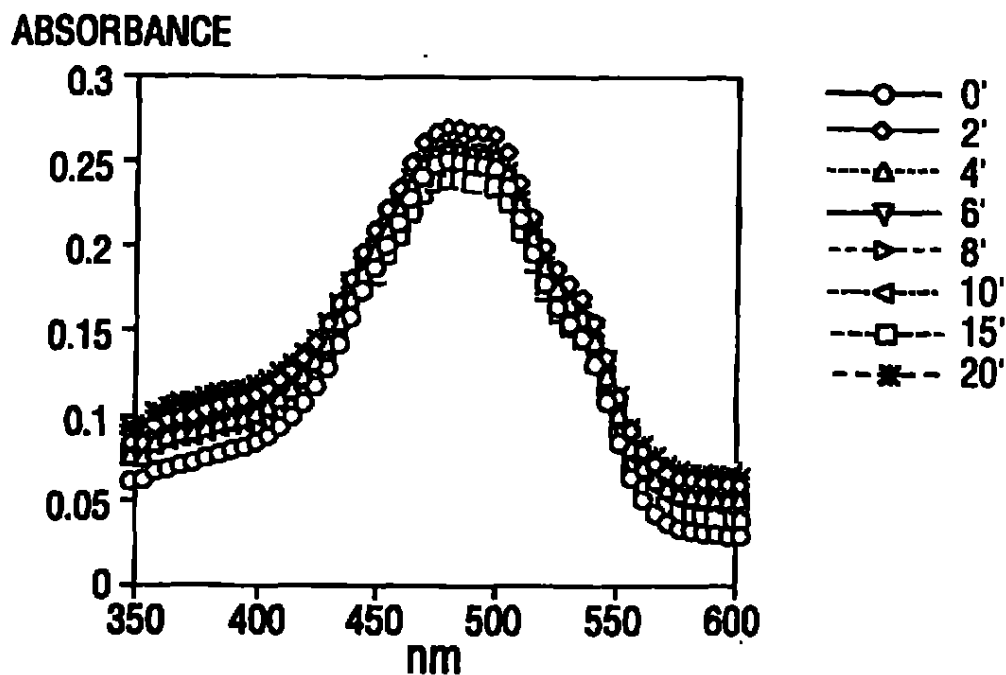


FIG. 5A

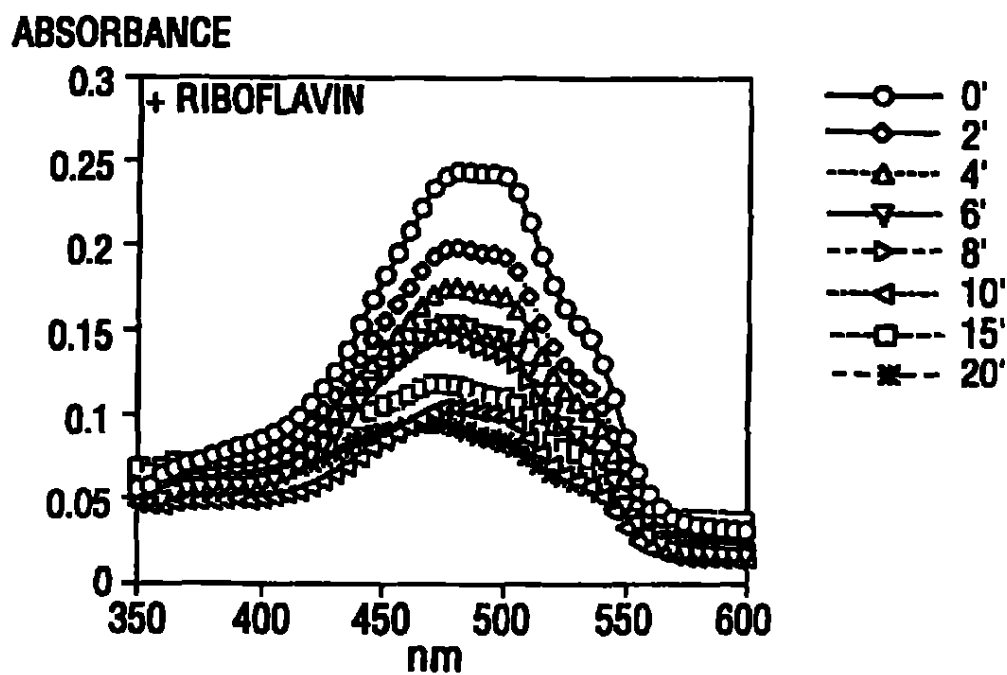


FIG. 5B

42

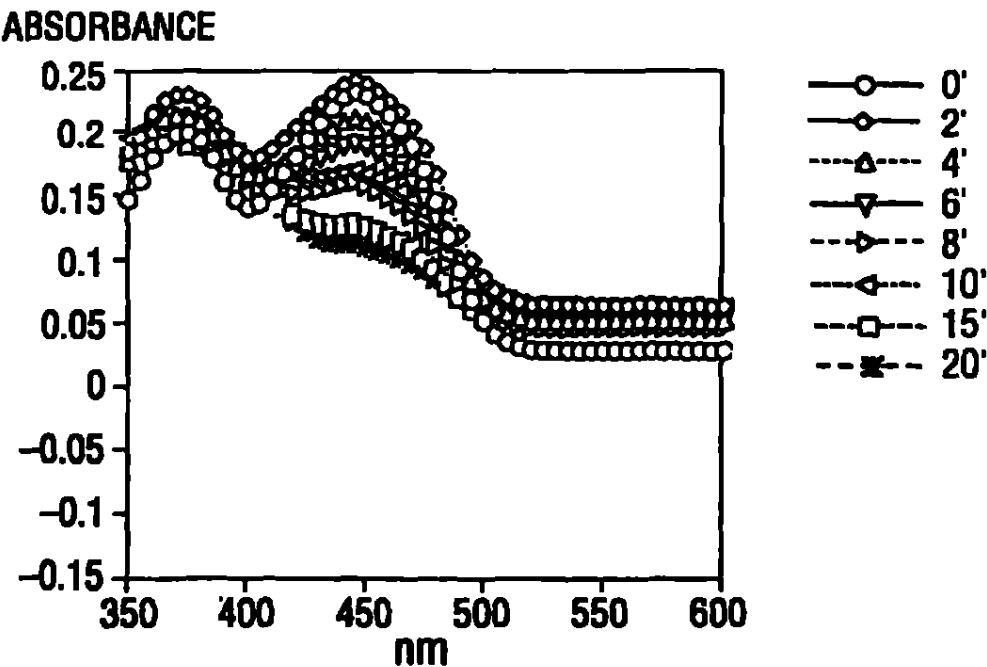


FIG. 6A

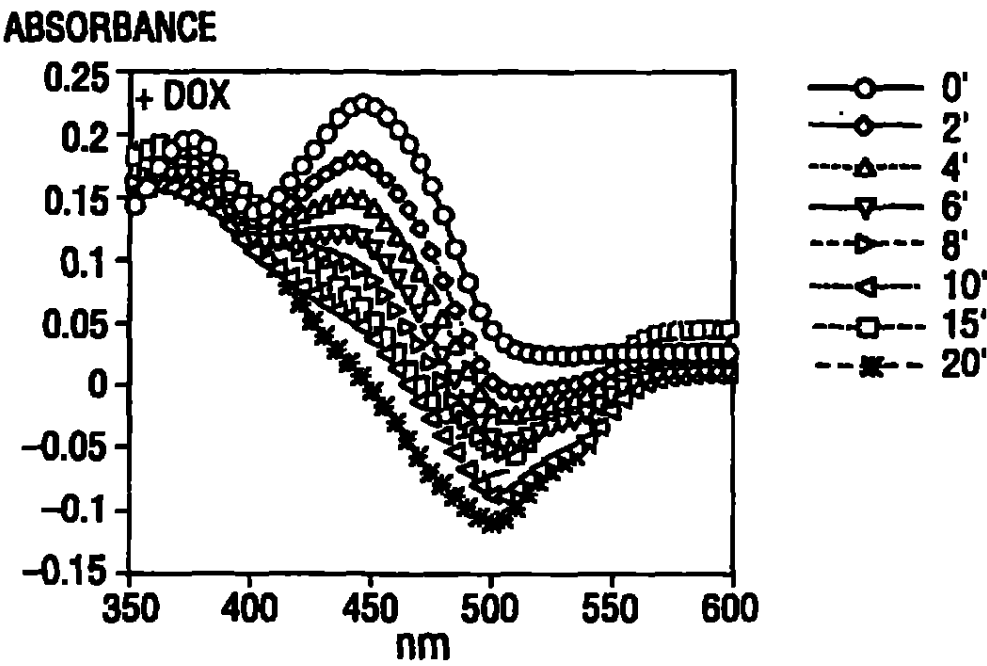


FIG. 6B

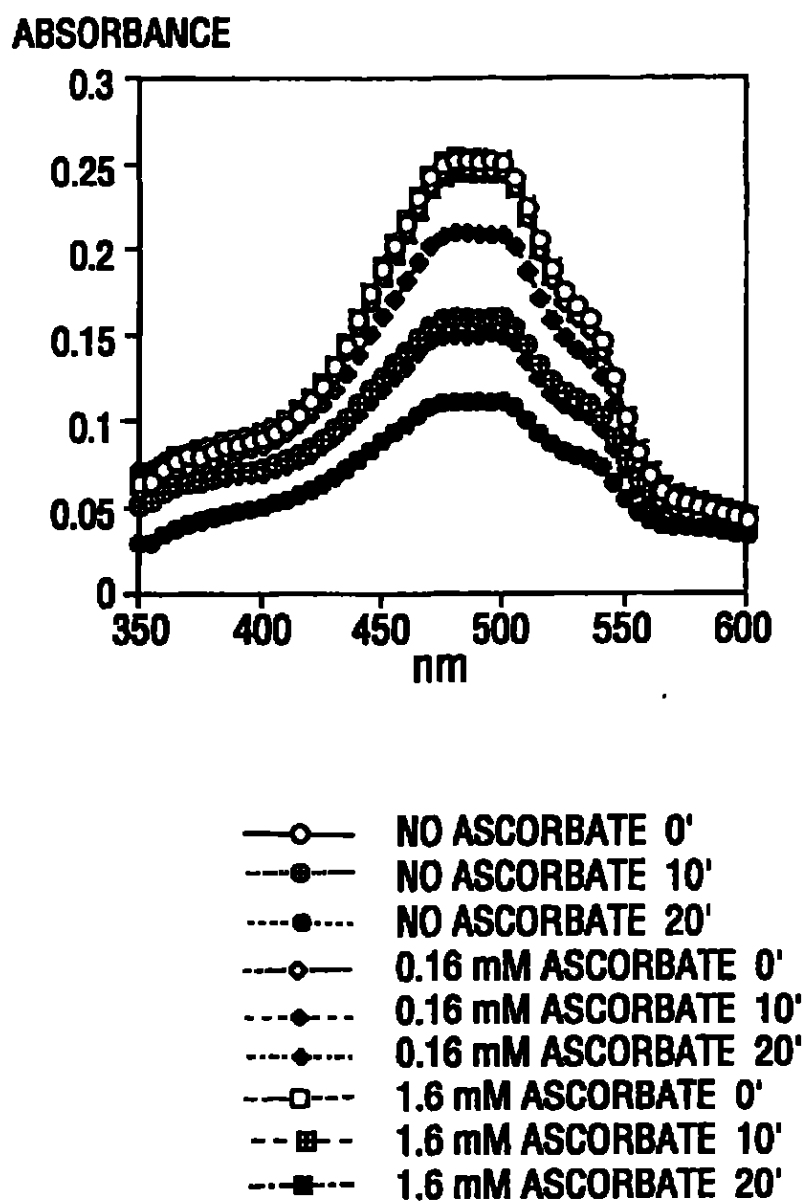


FIG. 7

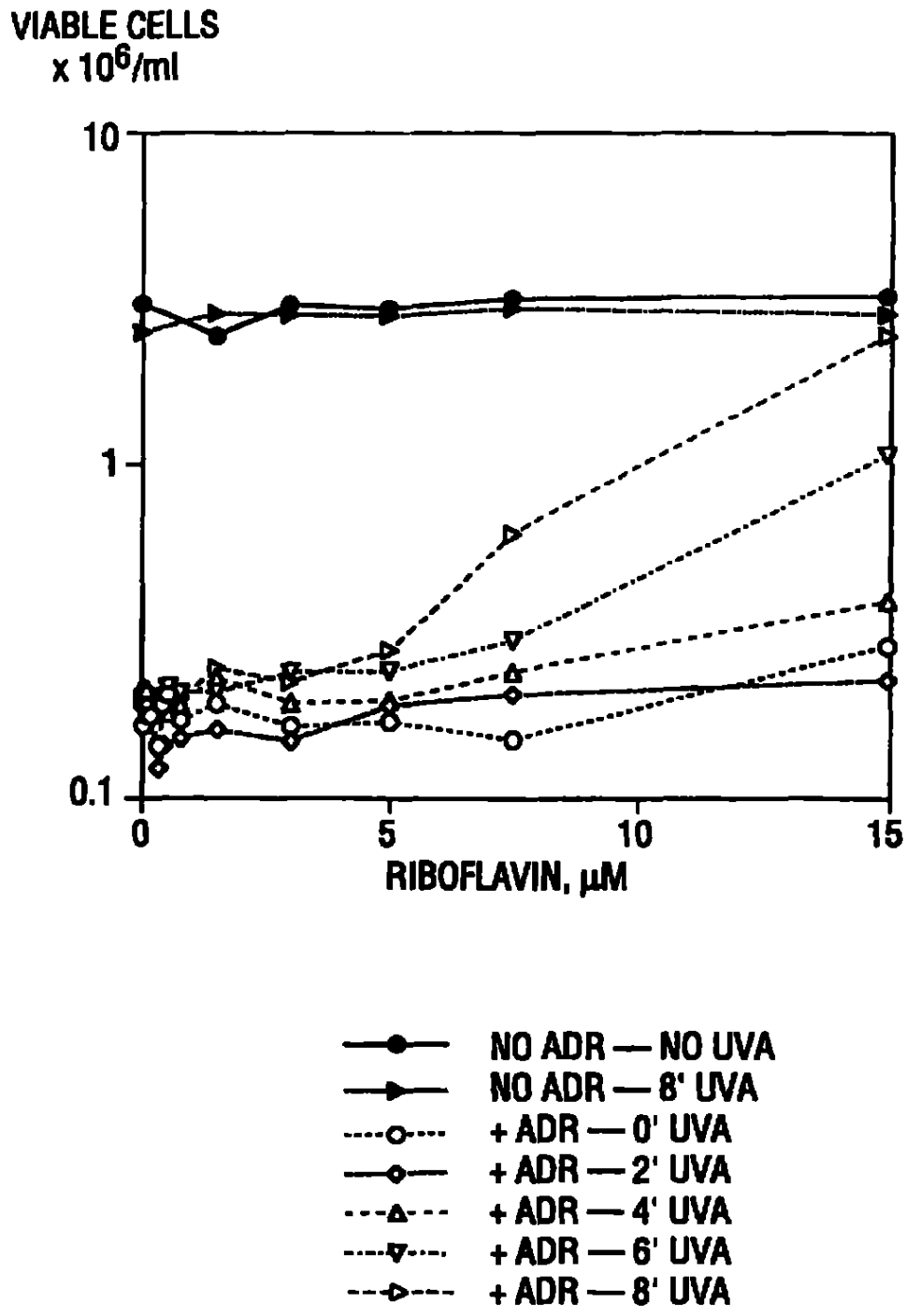


FIG. 8

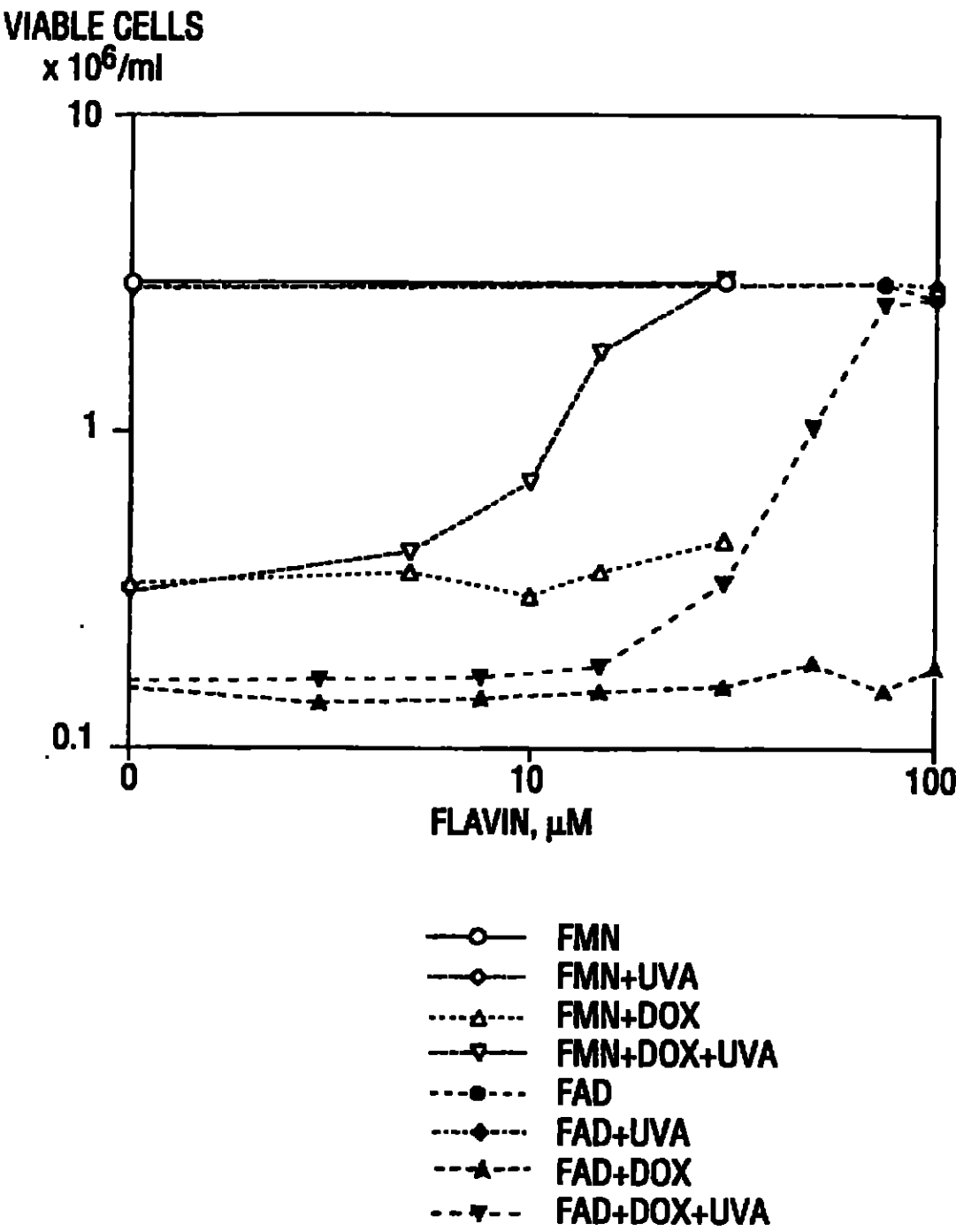


FIG. 9

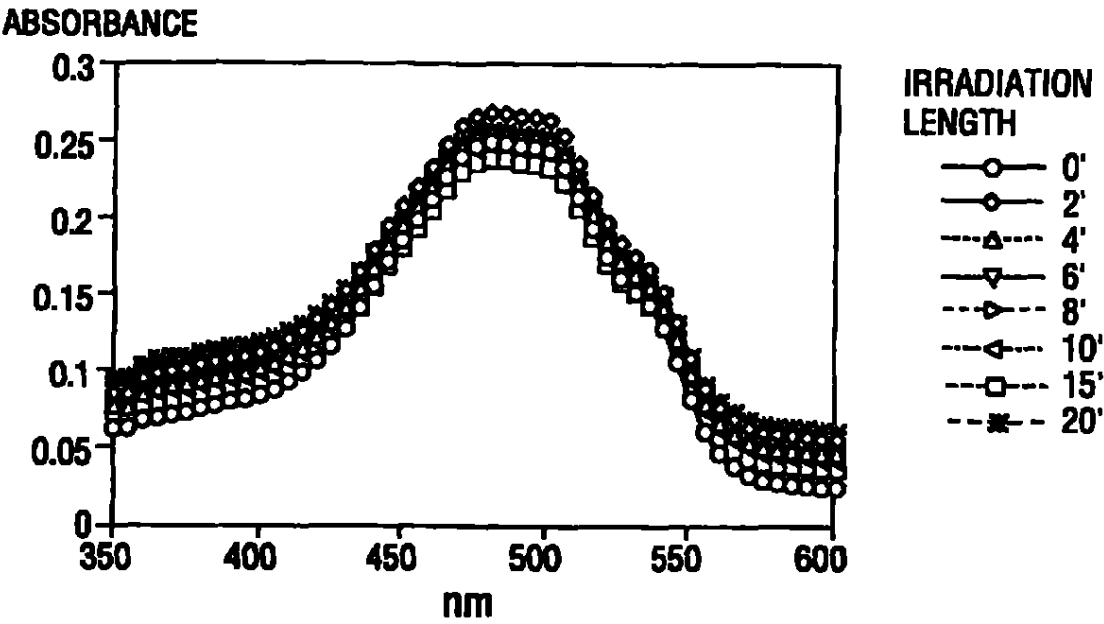


FIG. 10A

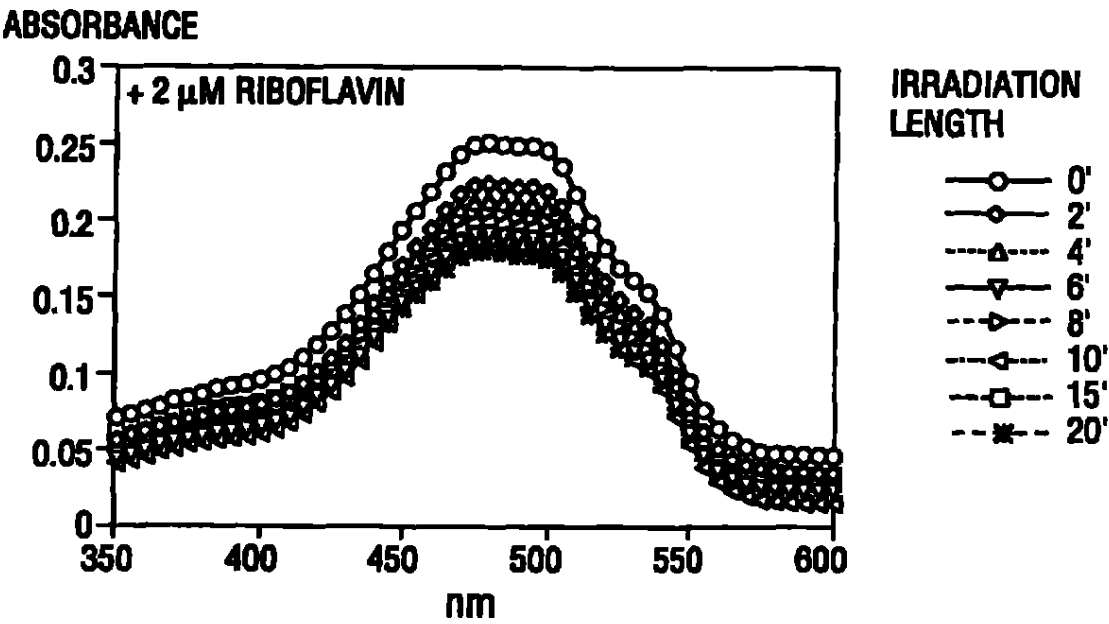


FIG. 10B

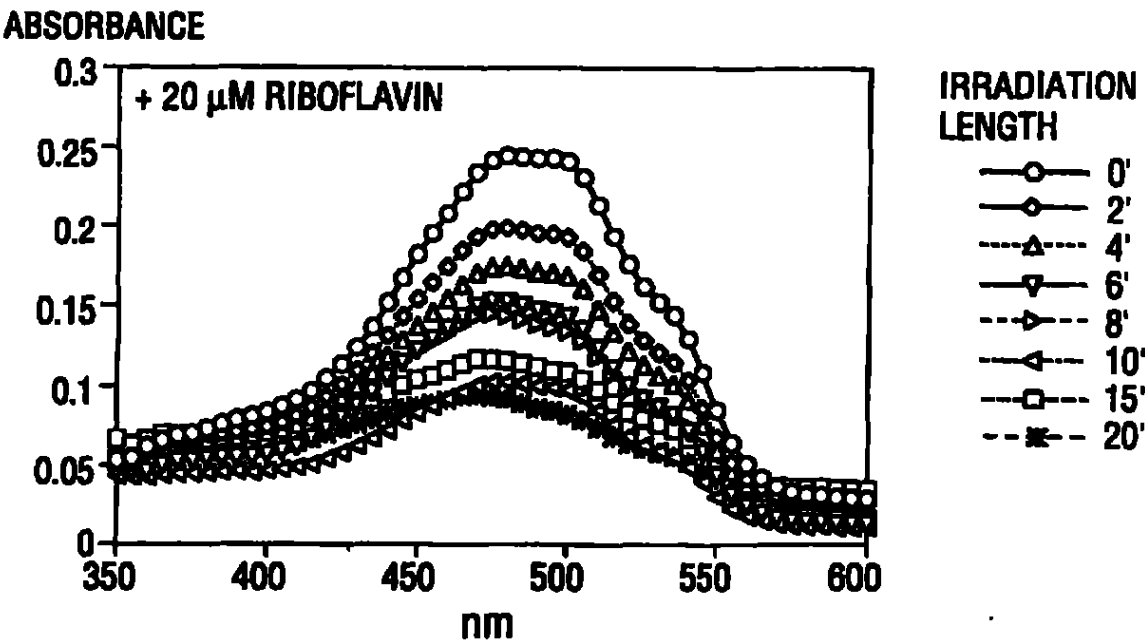
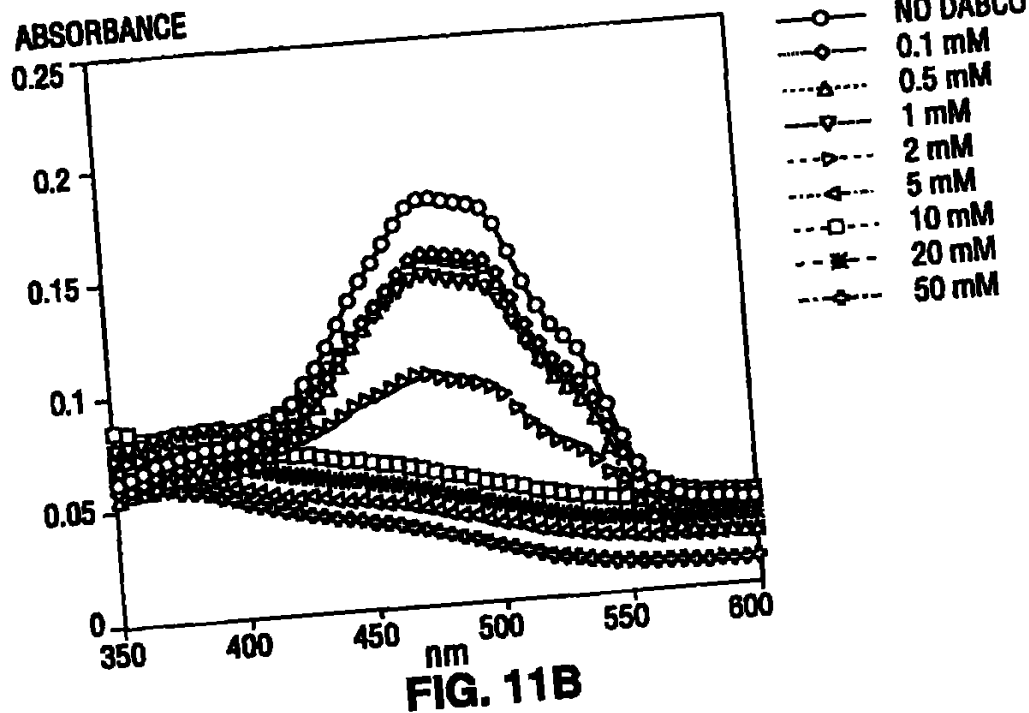
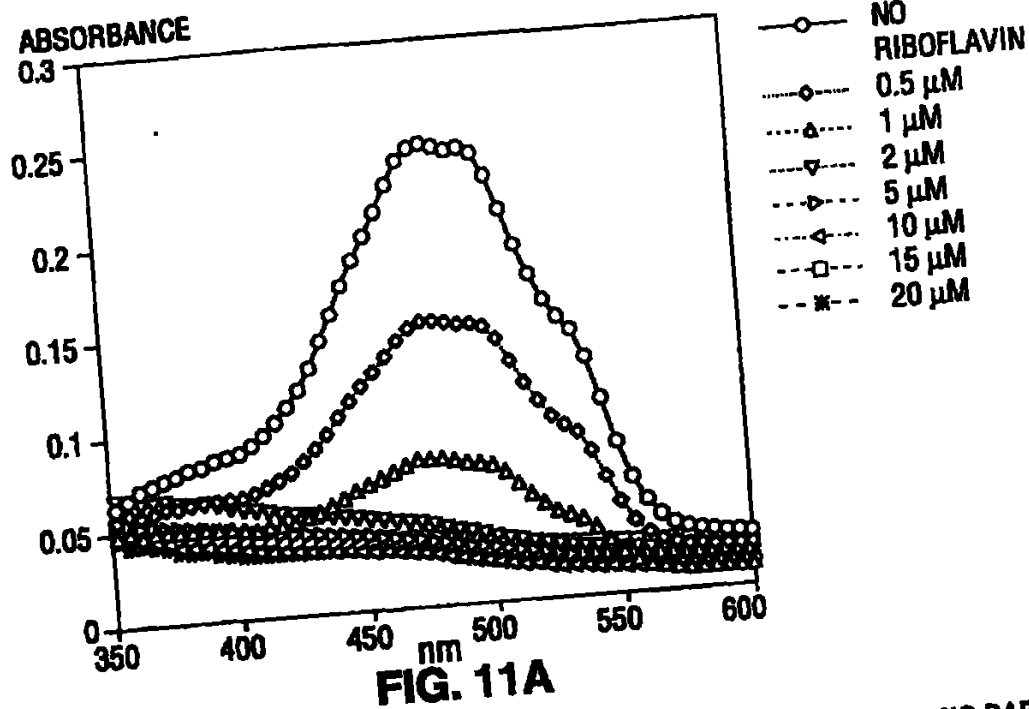


FIG. 10C



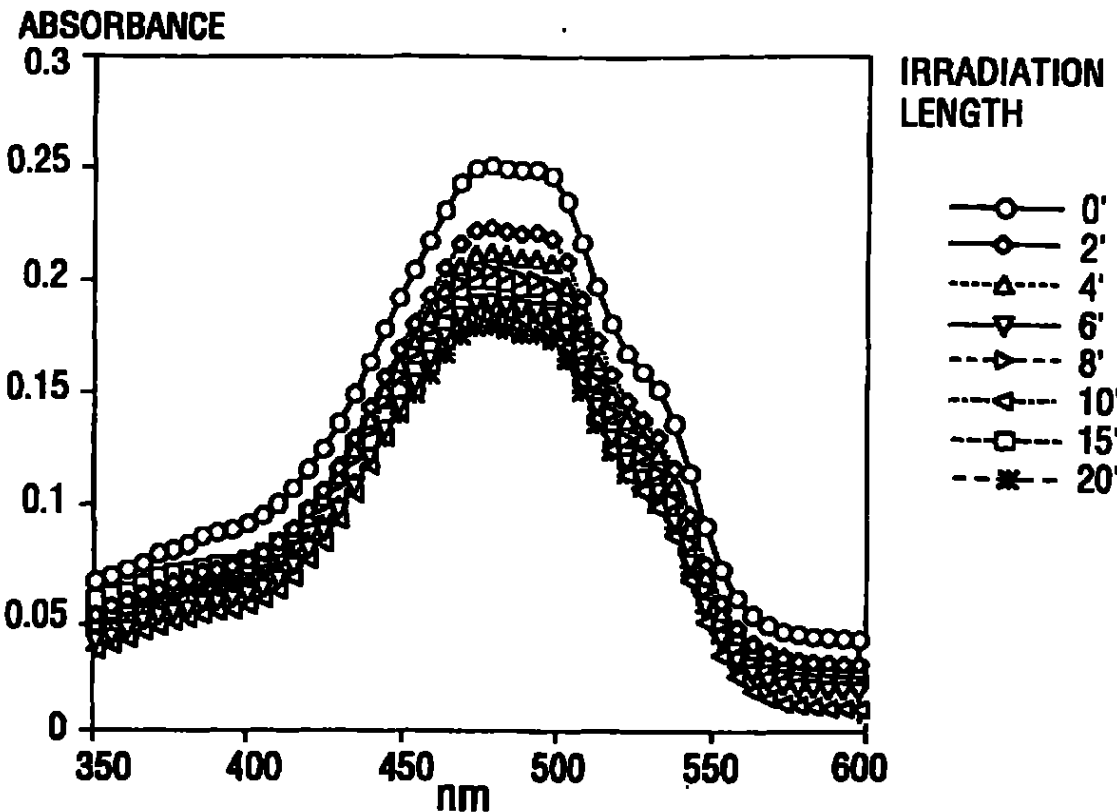


FIG. 12A

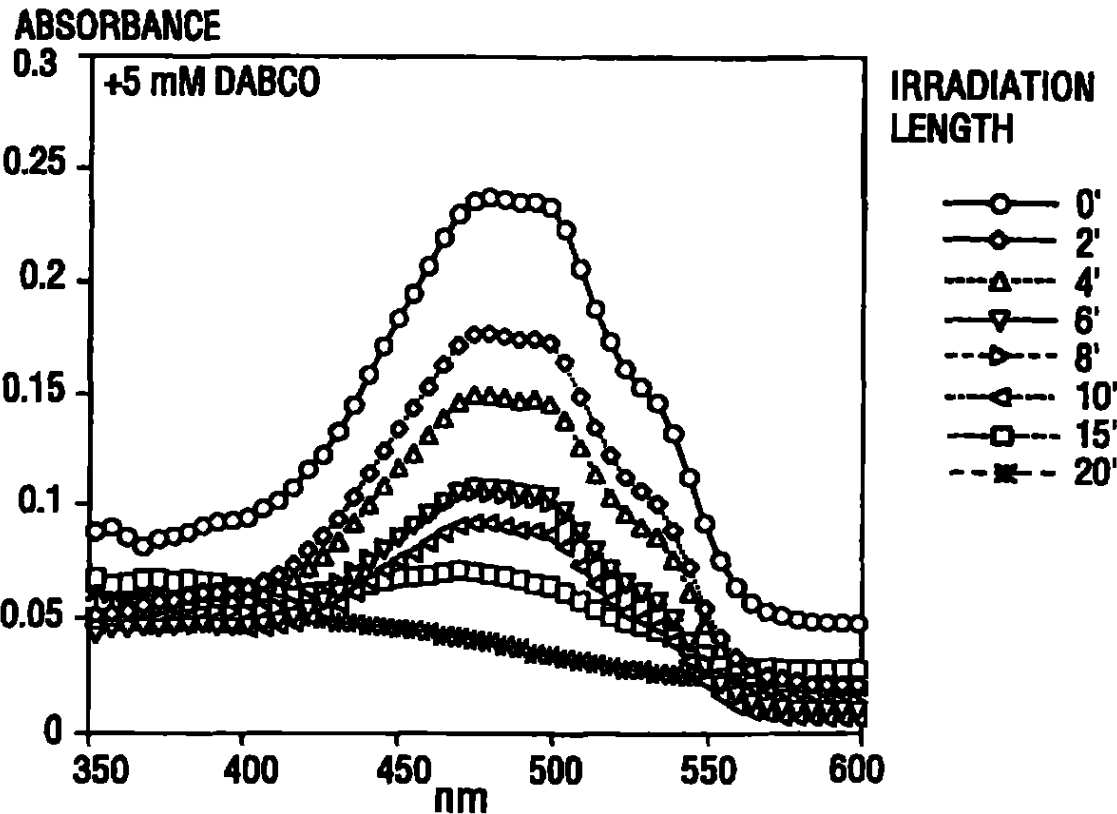


FIG. 12B

59
56

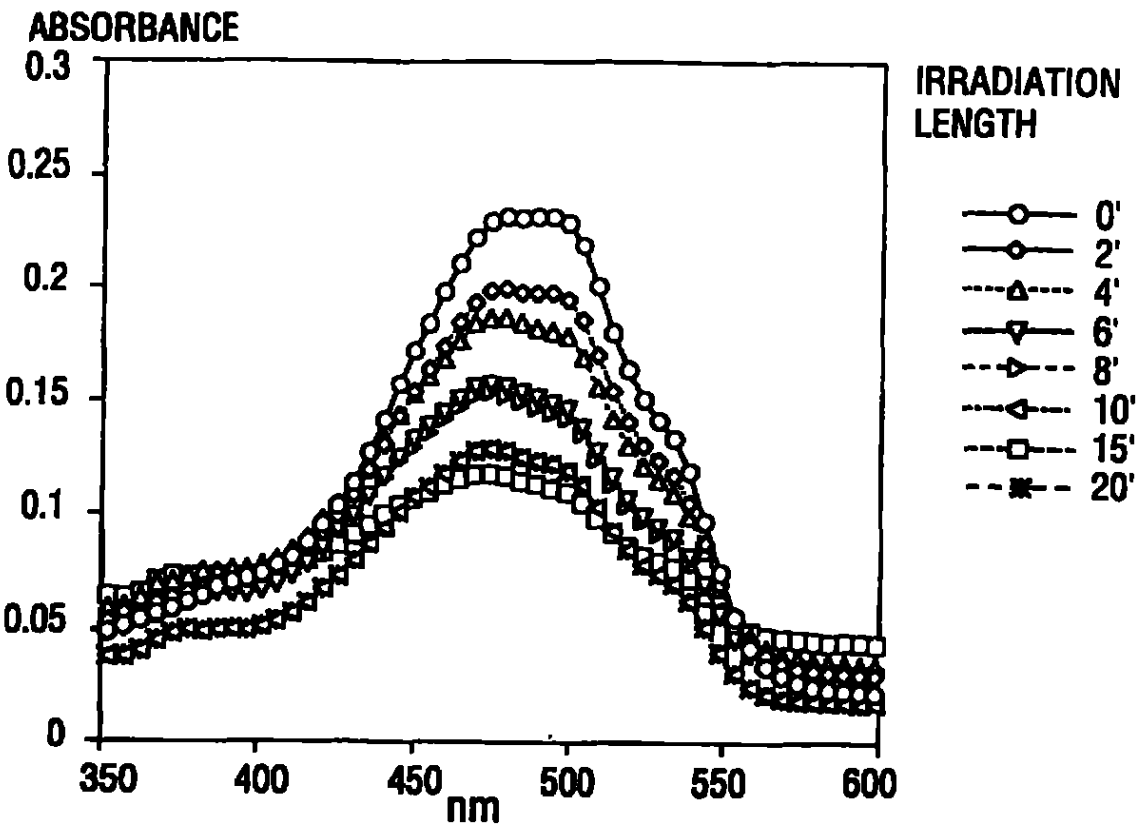


FIG. 13A

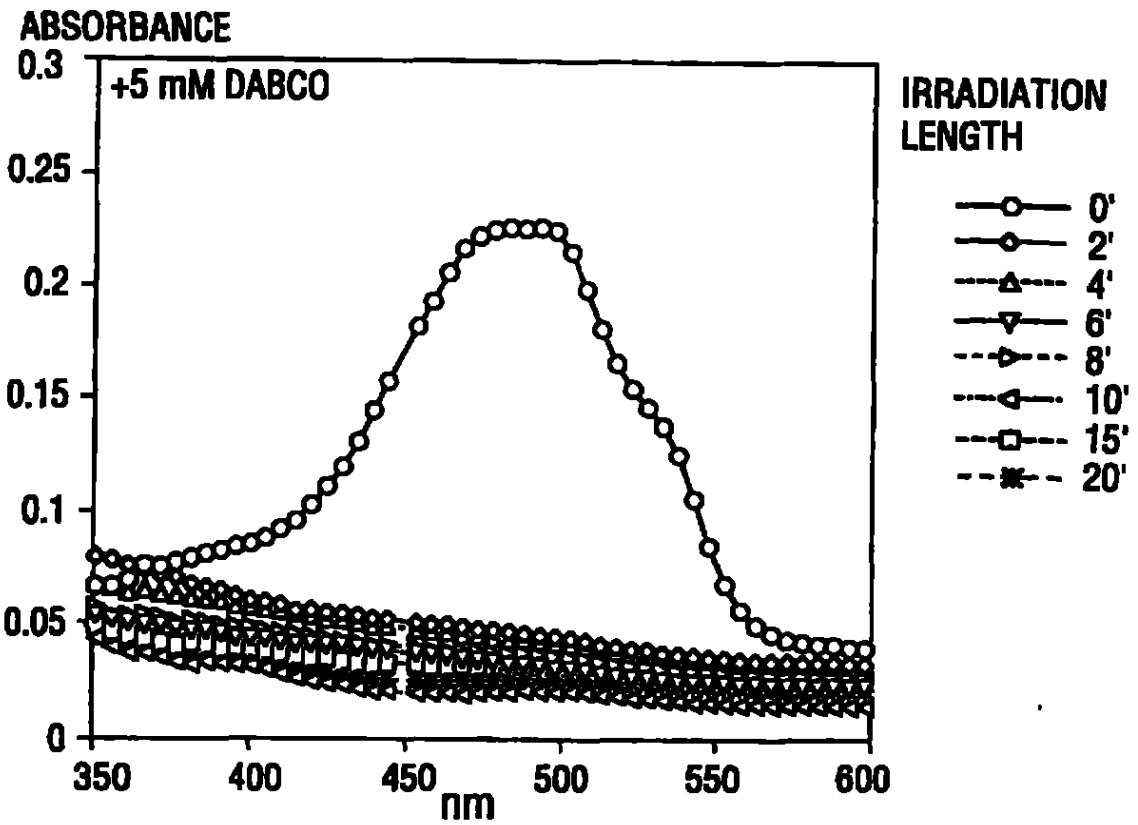


FIG. 13B

51

60
57

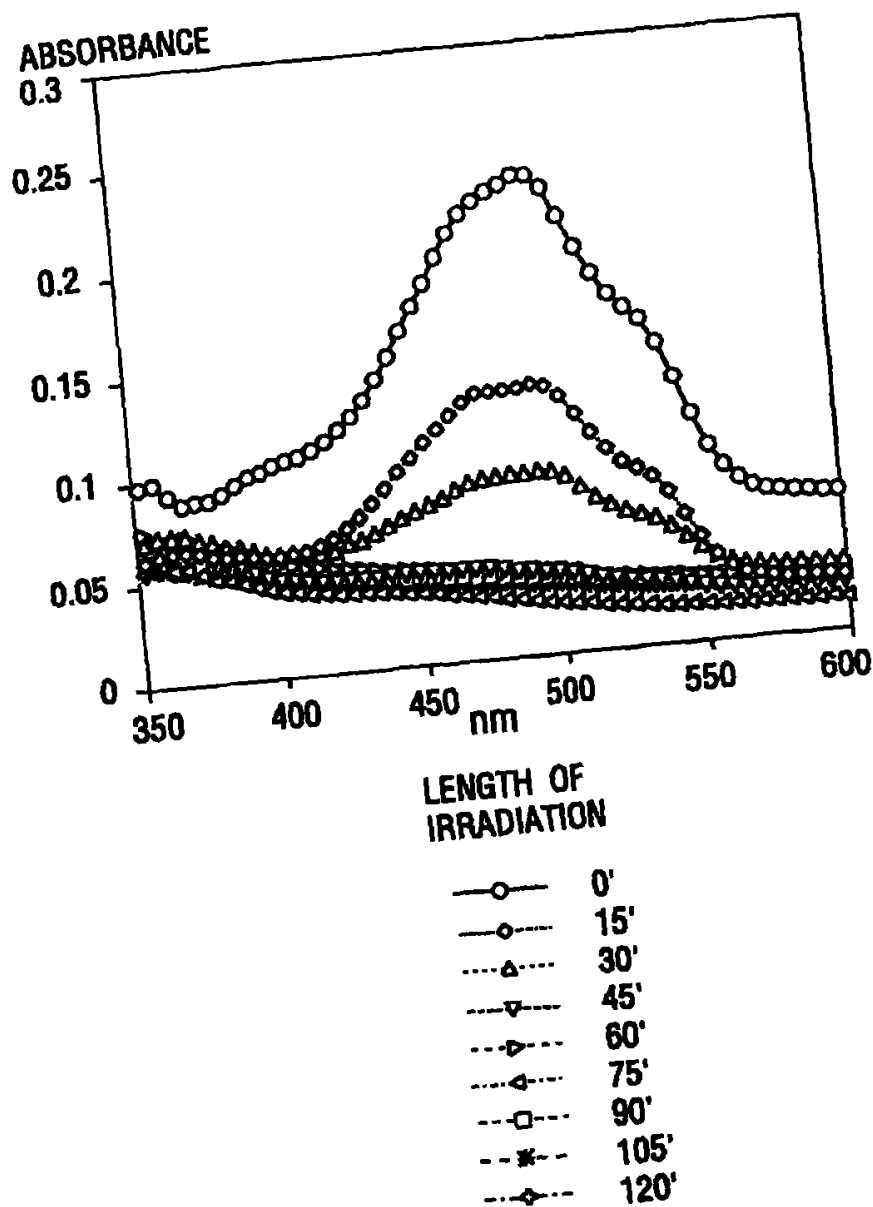


FIG. 13C

52

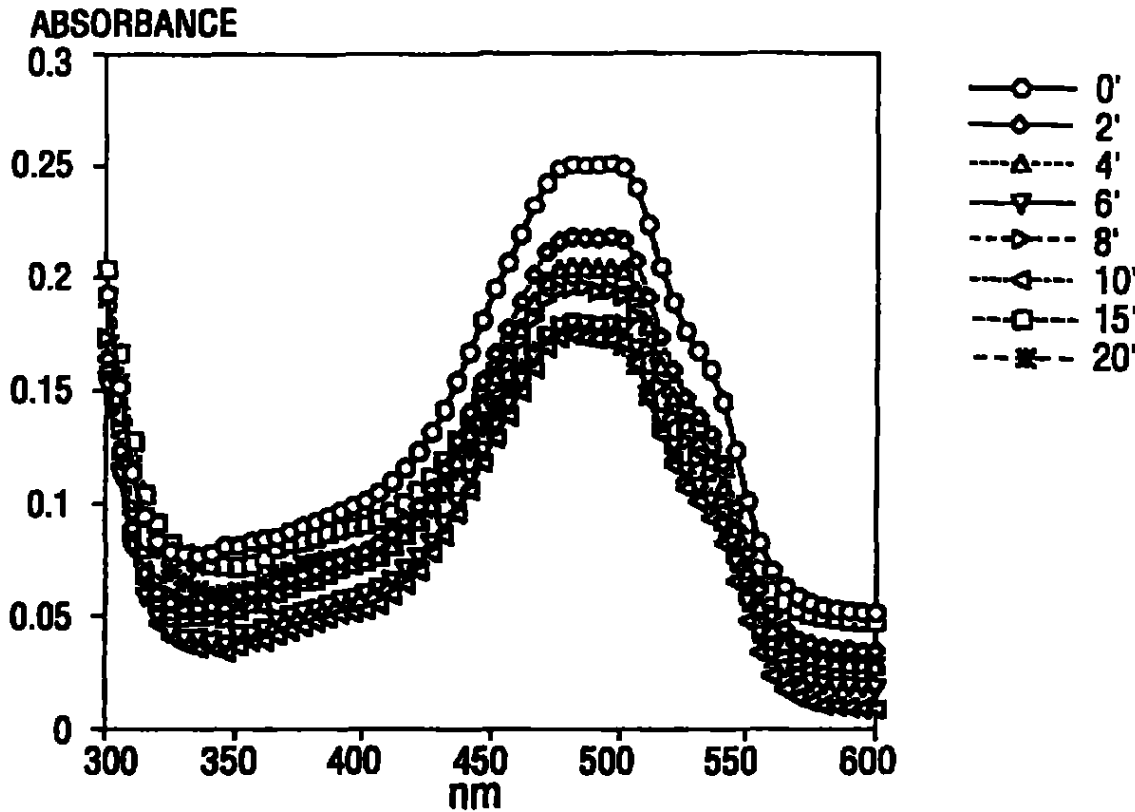


FIG. 14A

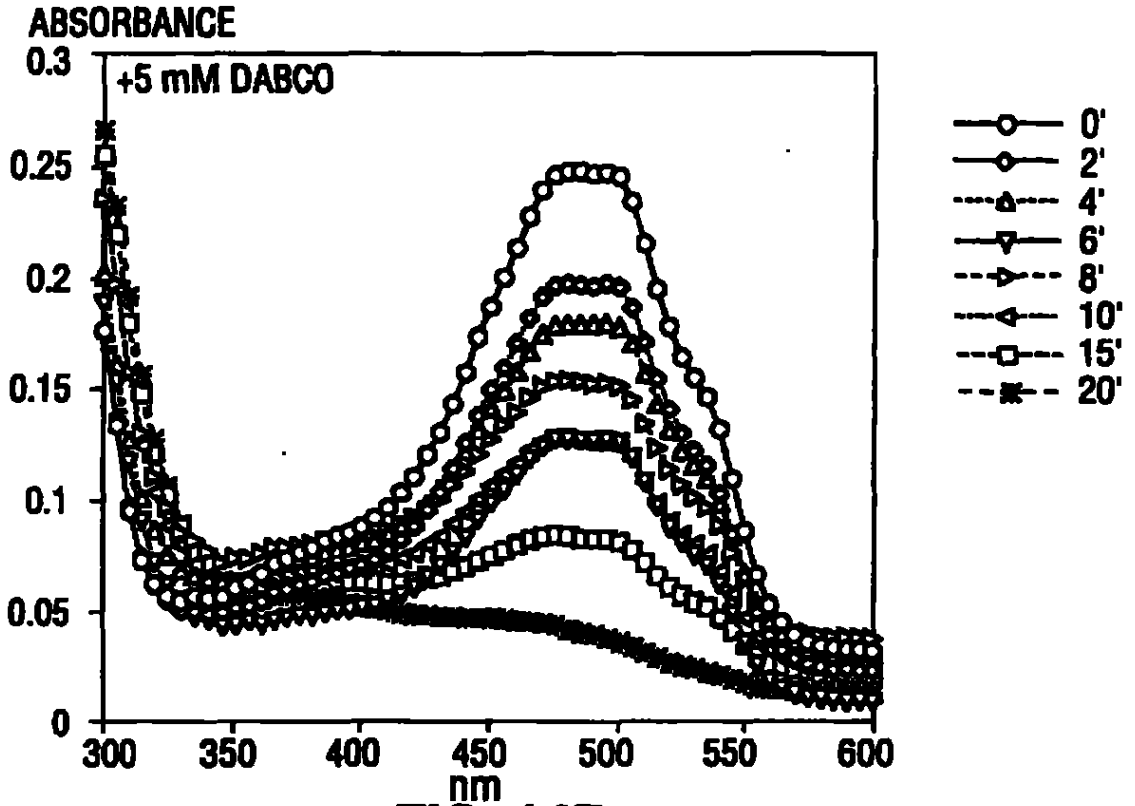


FIG. 14B

62
59

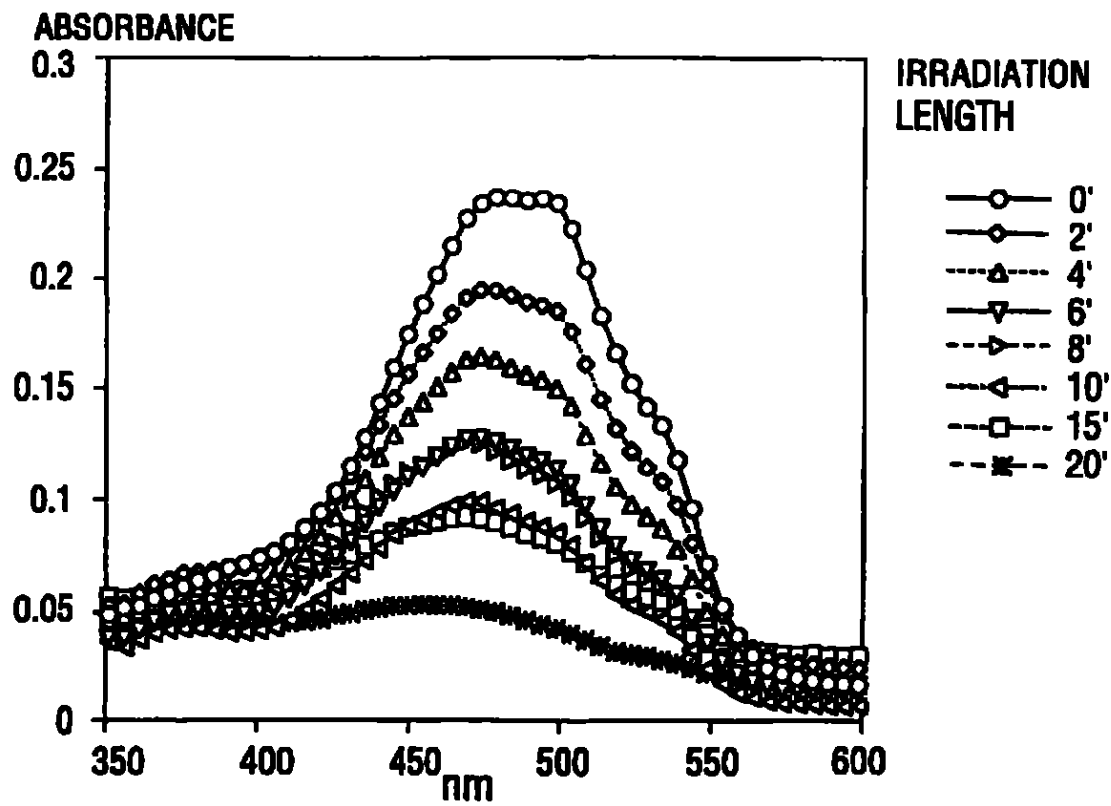


FIG. 15A

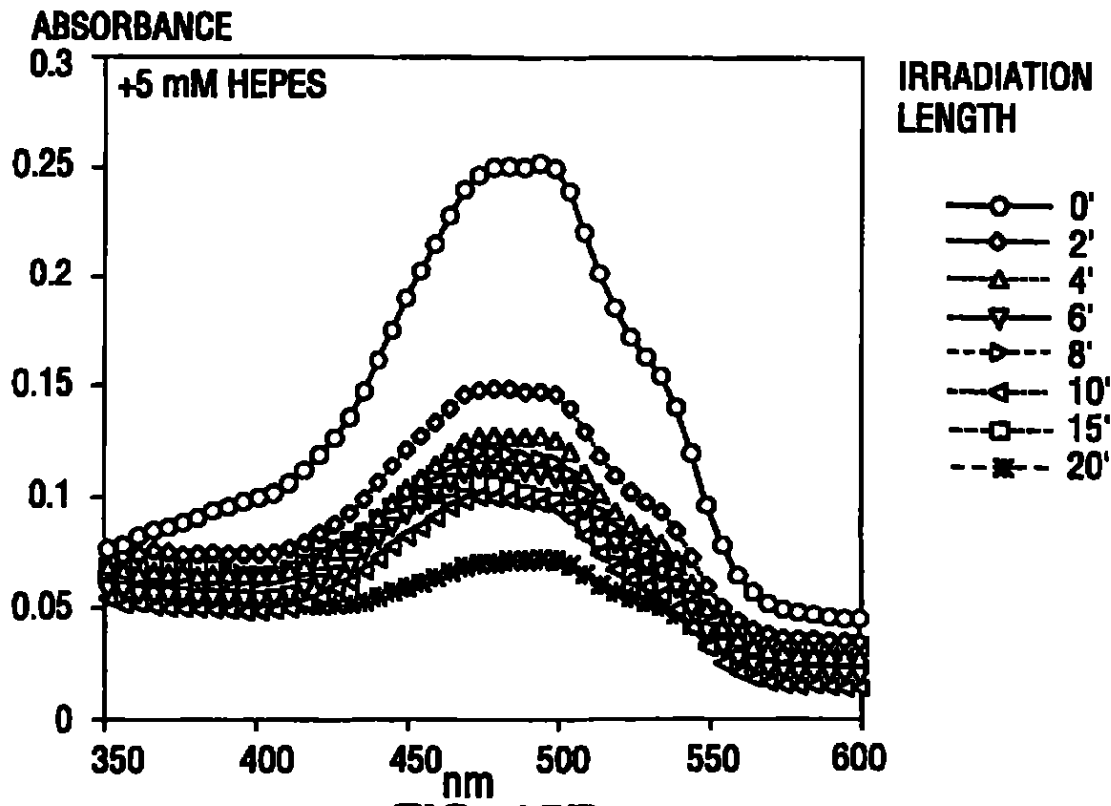


FIG. 15B

54

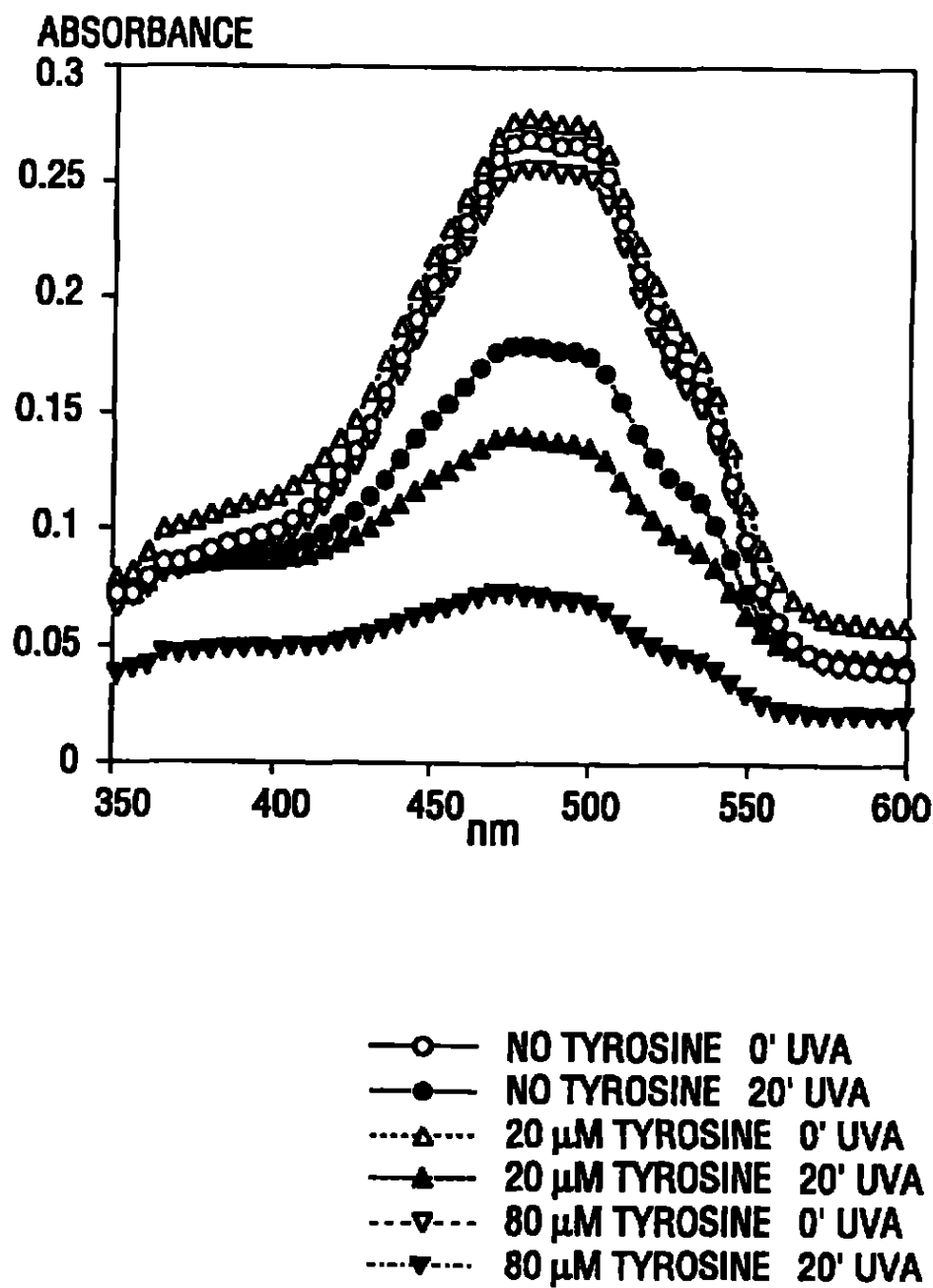


FIG. 16

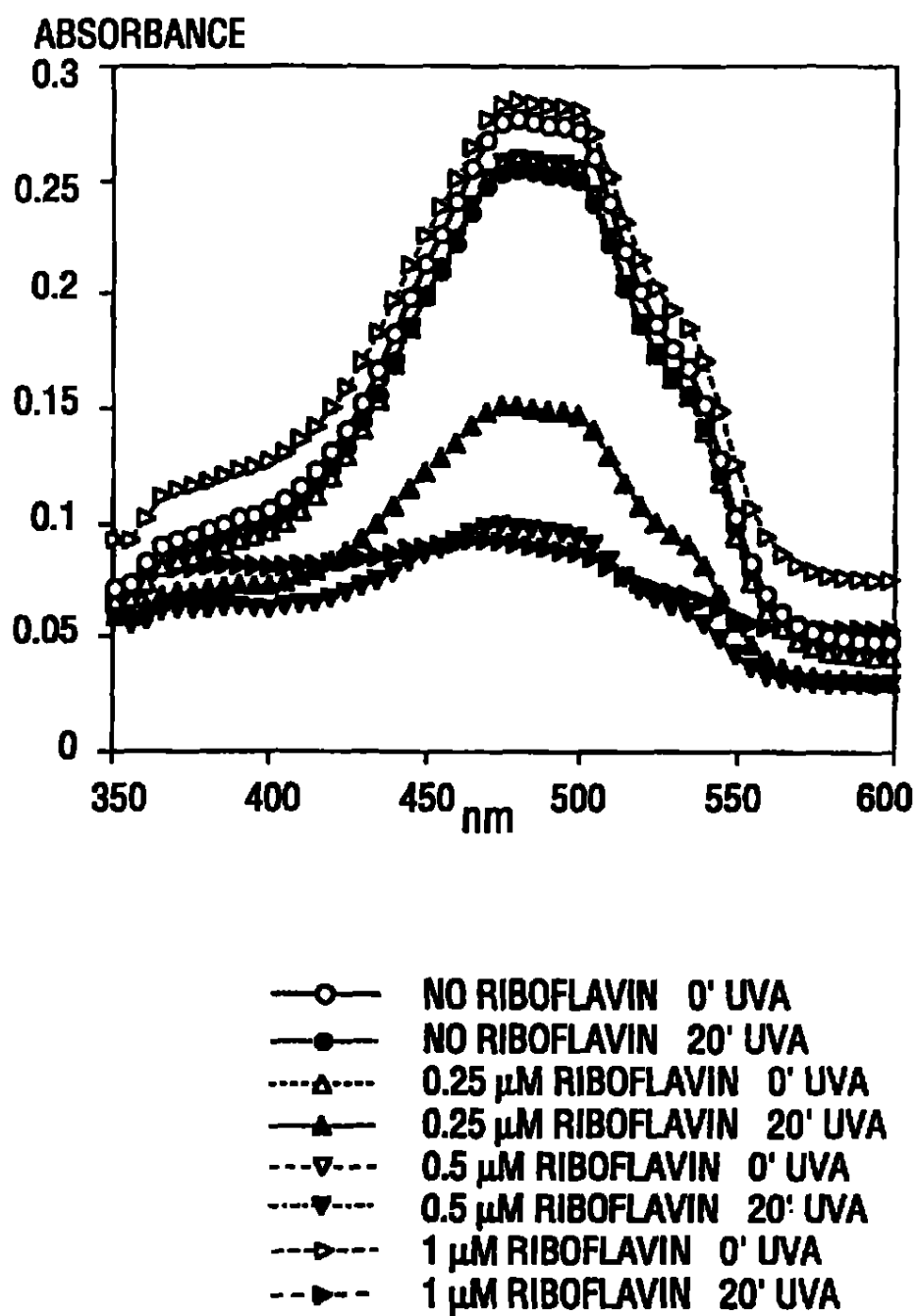


FIG. 17

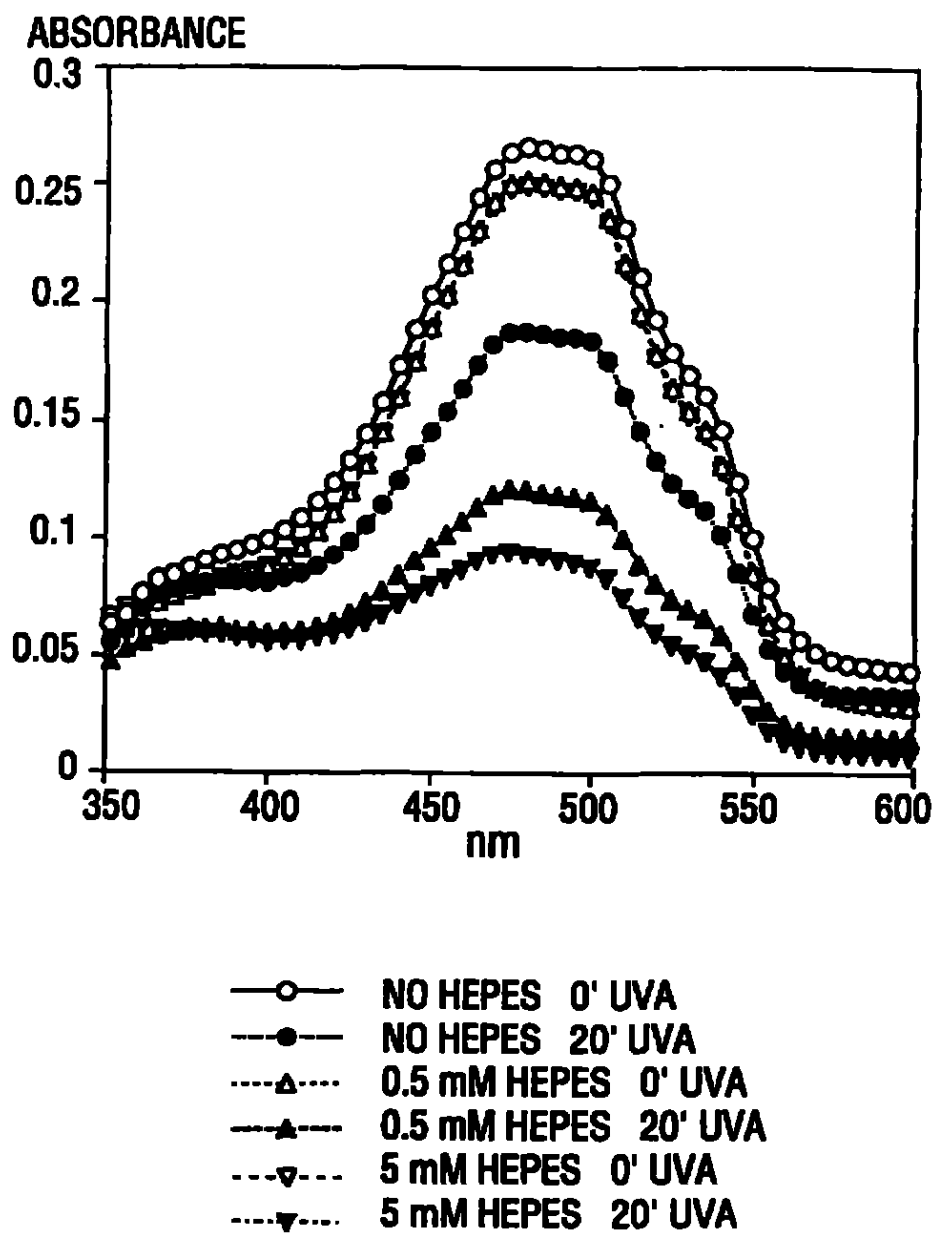
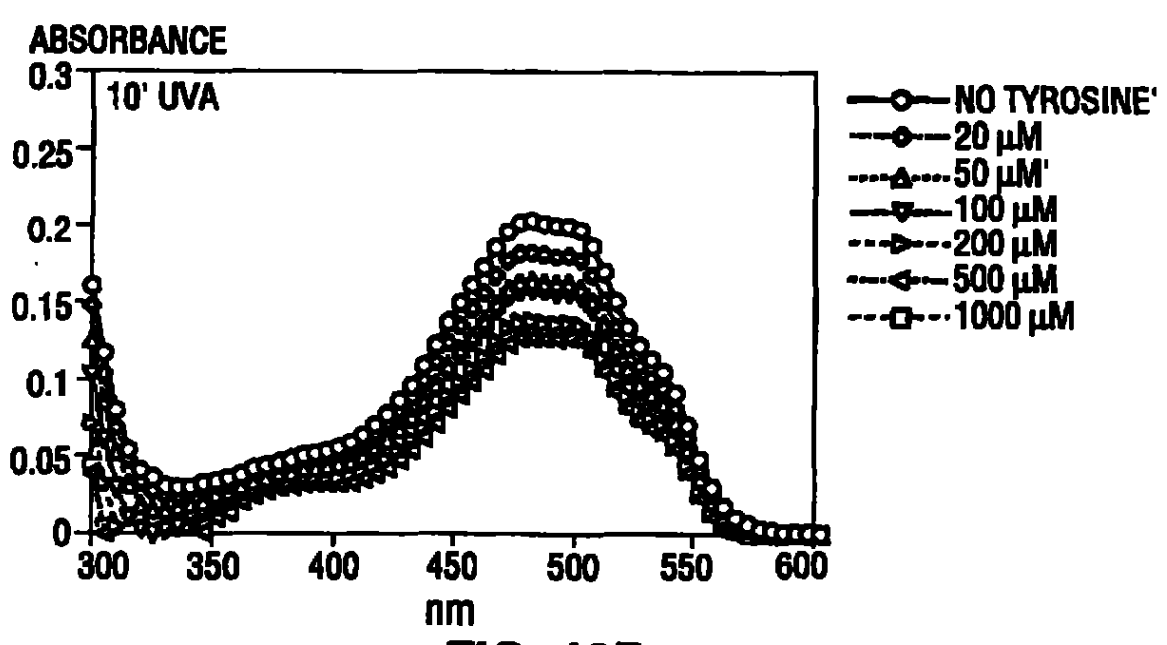
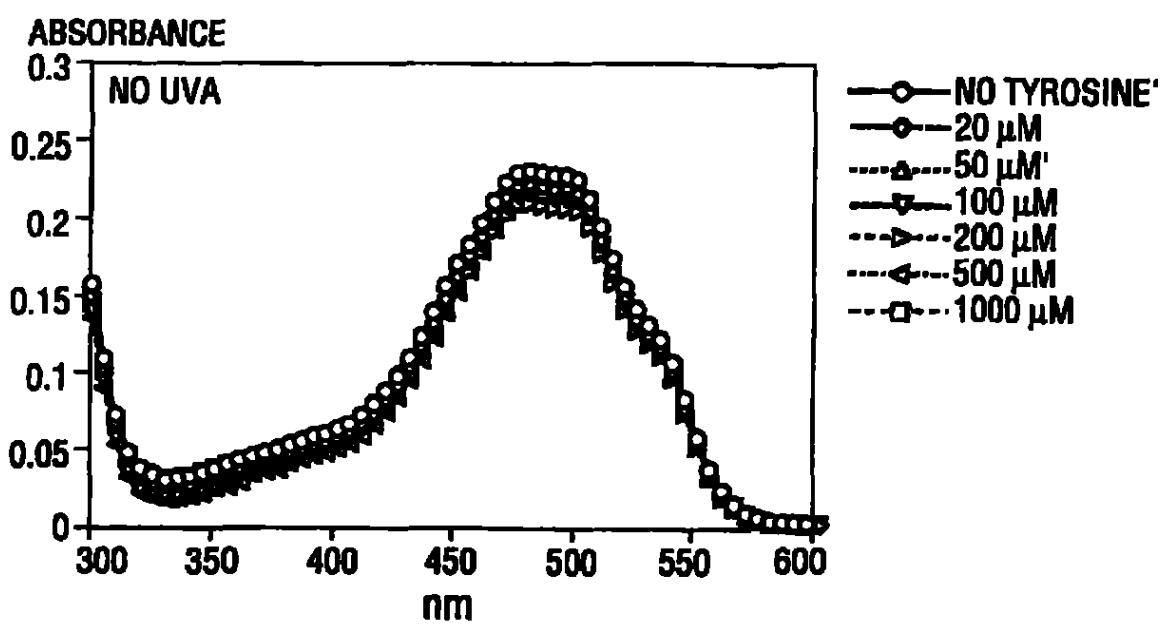


FIG. 18



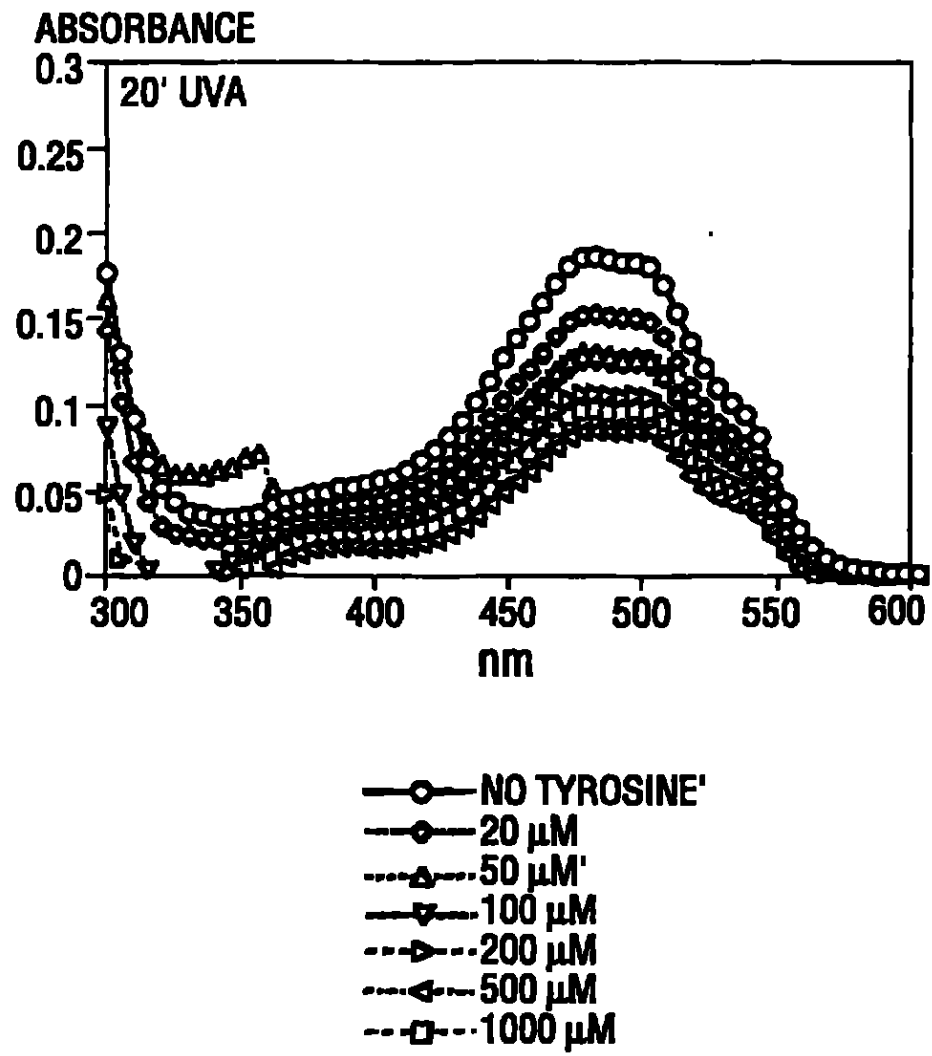


FIG. 19C

68
65

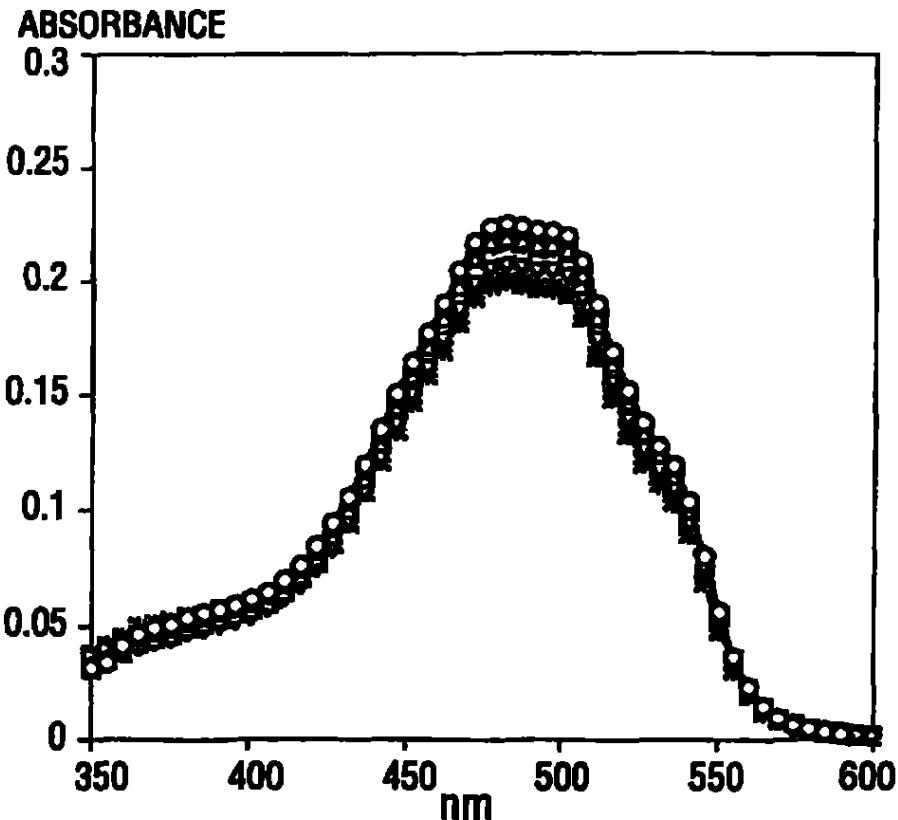


FIG. 20A

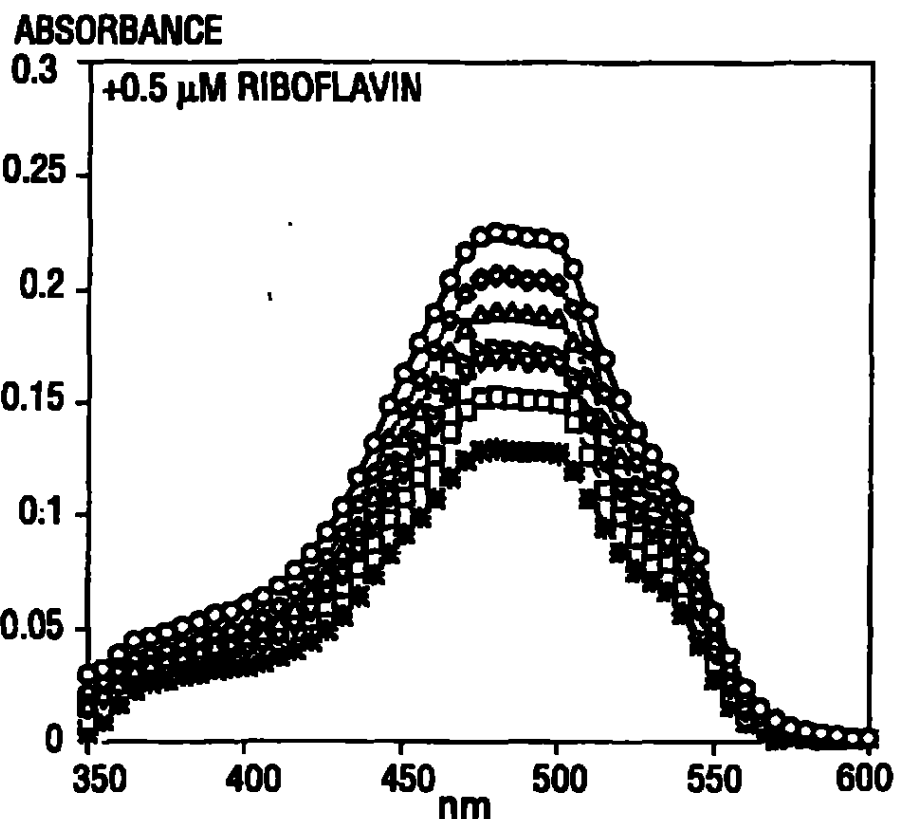


FIG. 20B

60

69
66

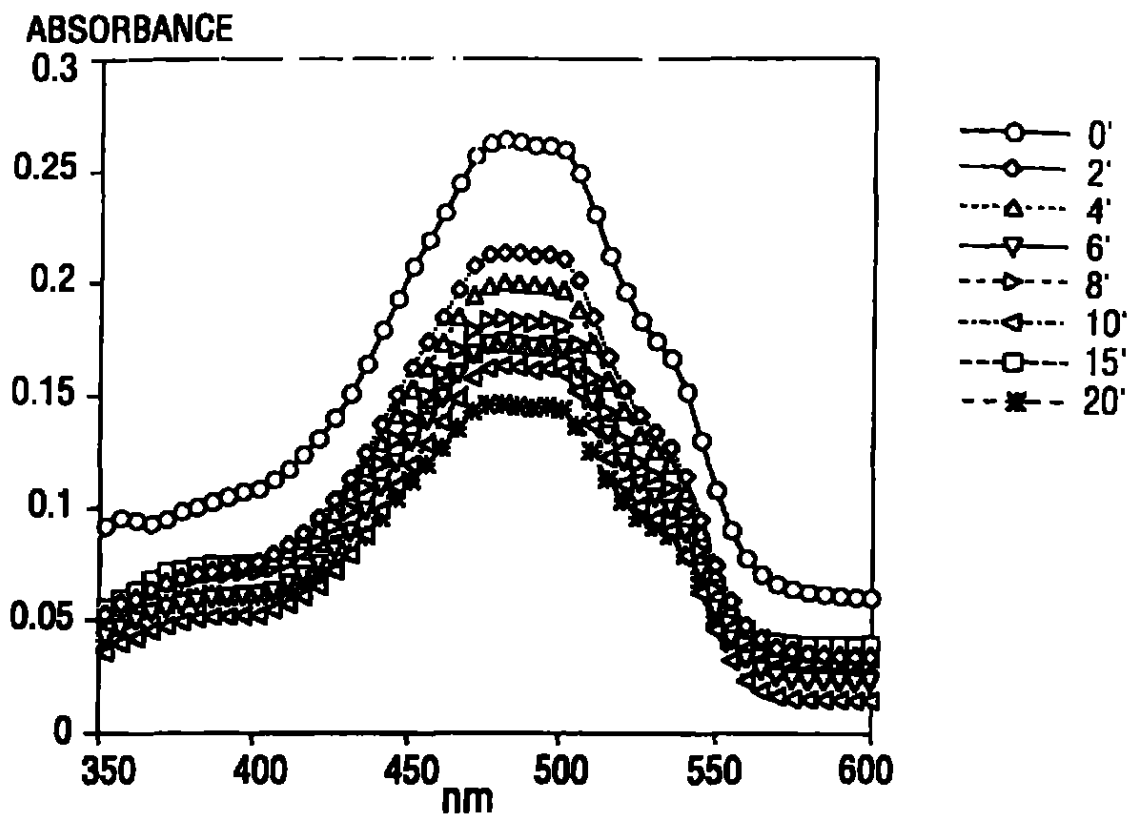


FIG. 21A

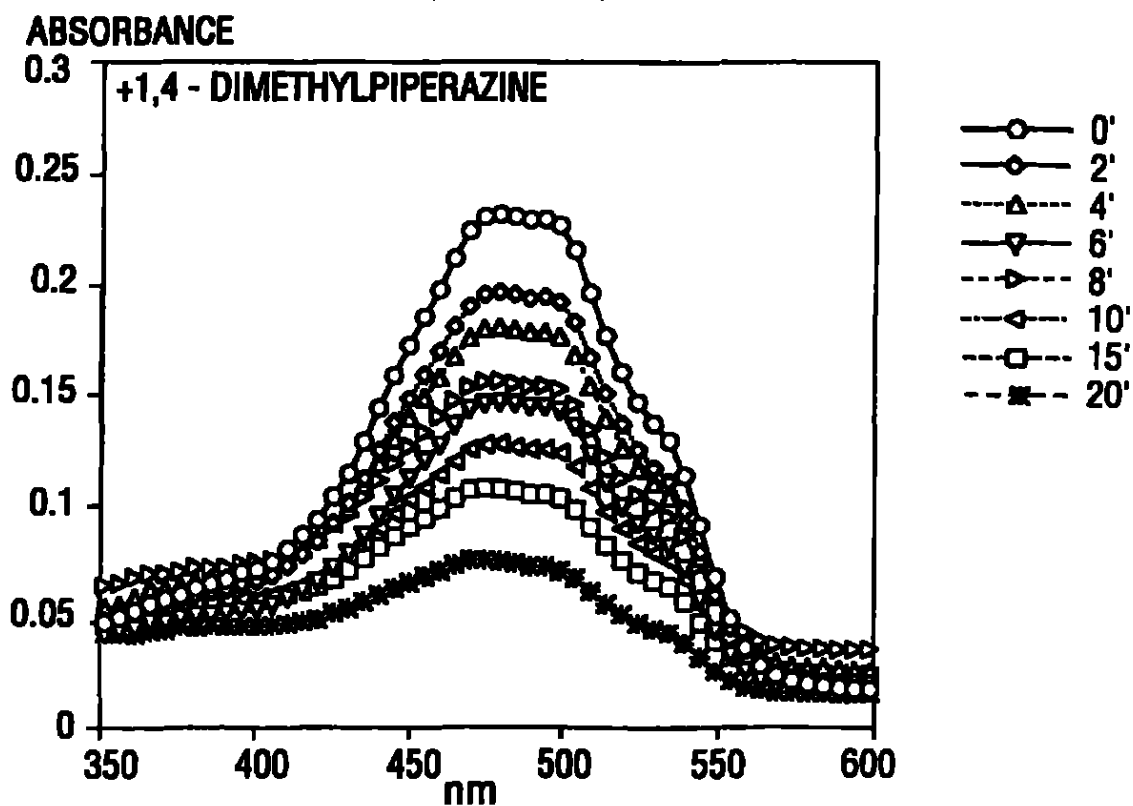


FIG. 21B

GI

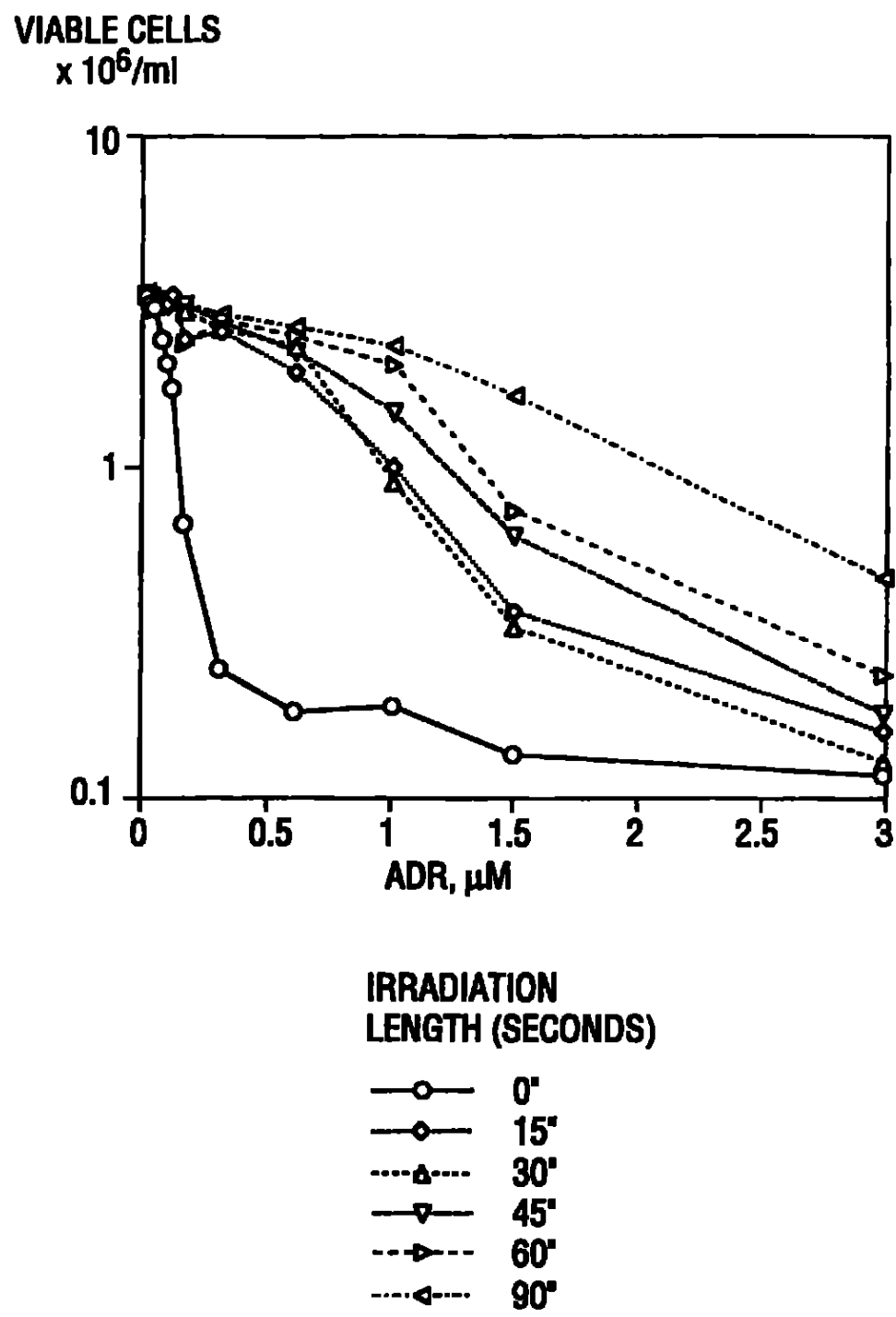


FIG. 22

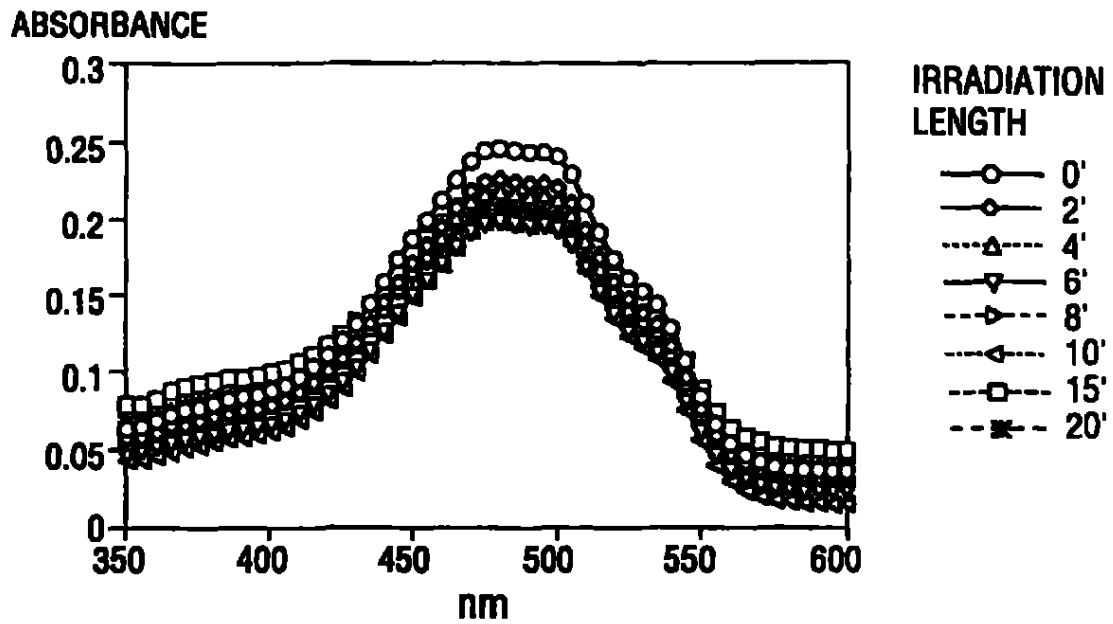


FIG. 23A

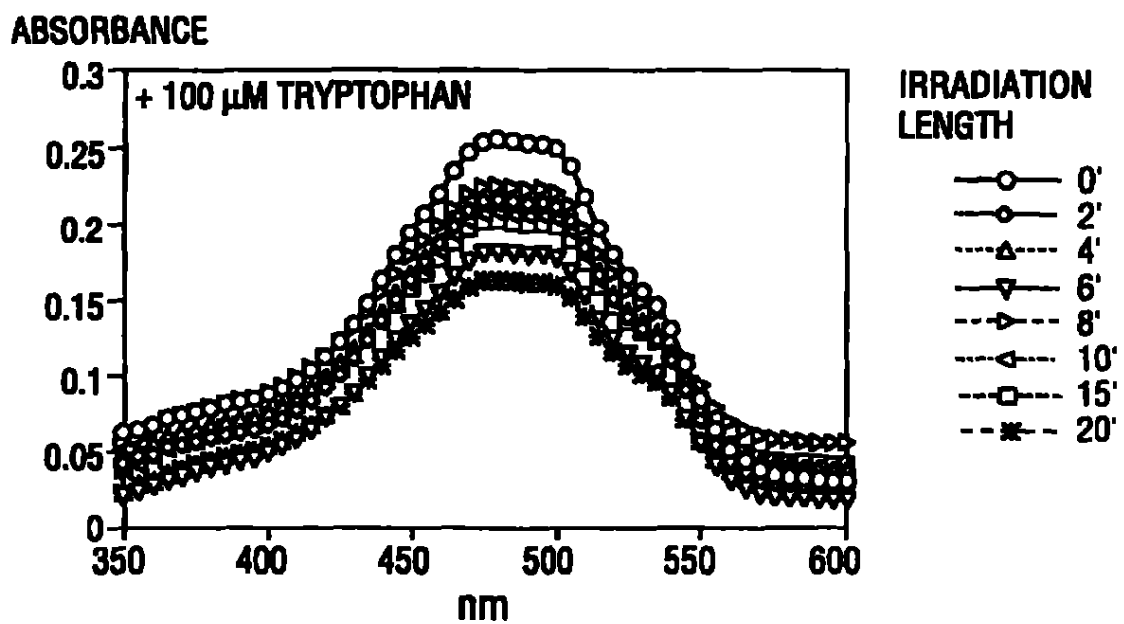


FIG. 23B

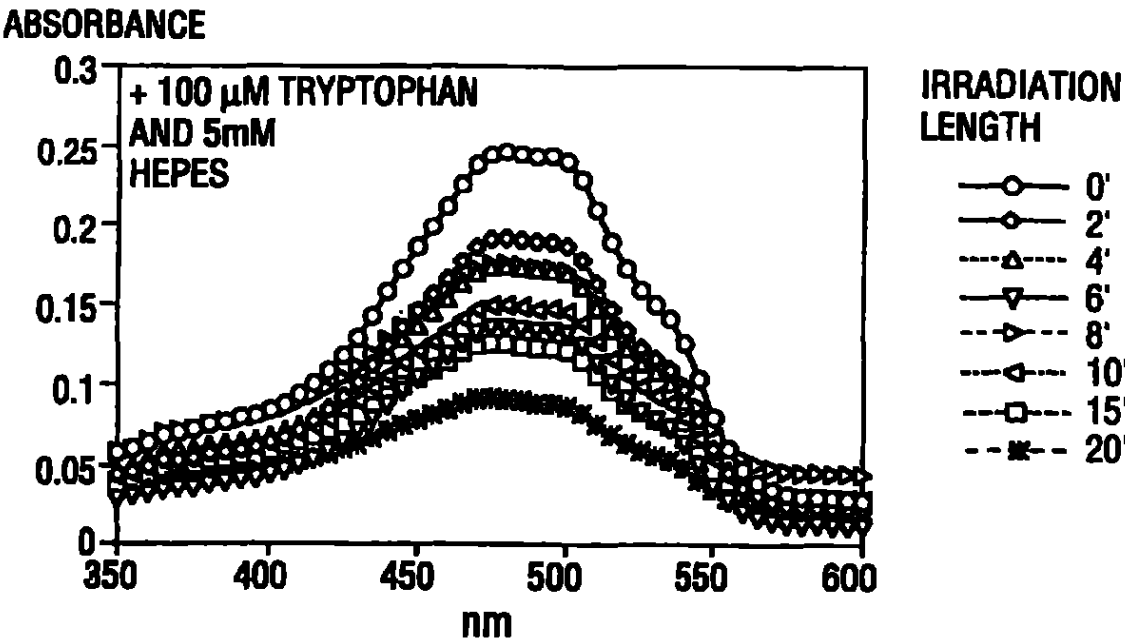


FIG. 23C

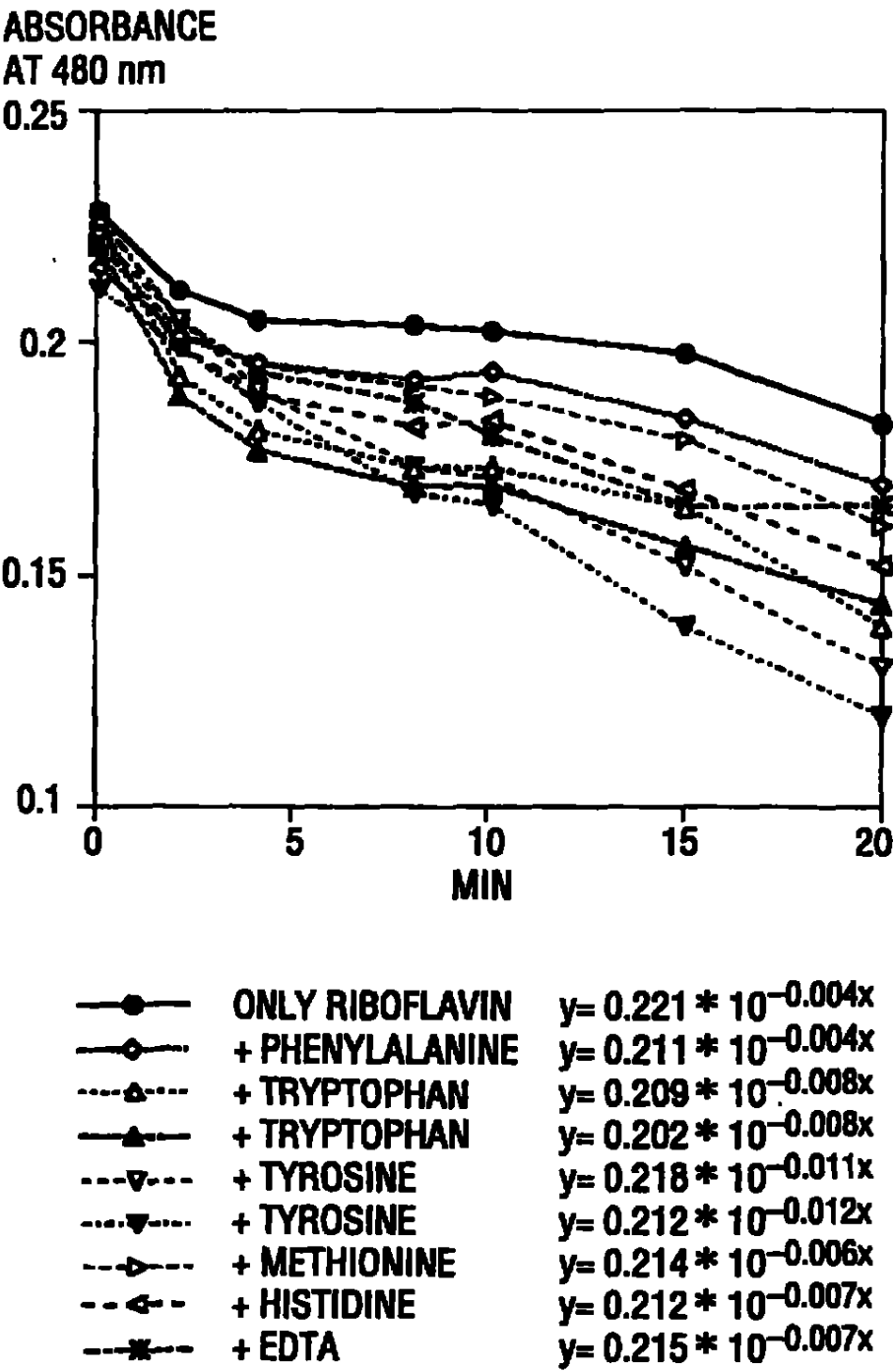


FIG. 24

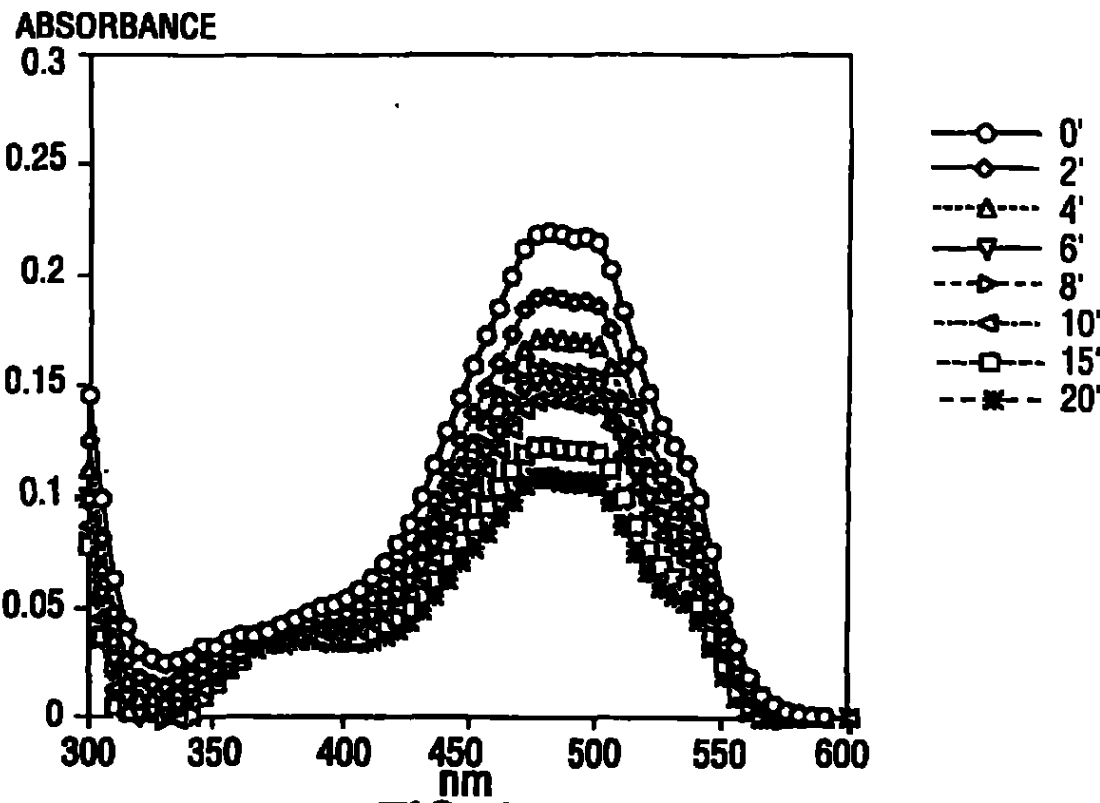


FIG. 25A

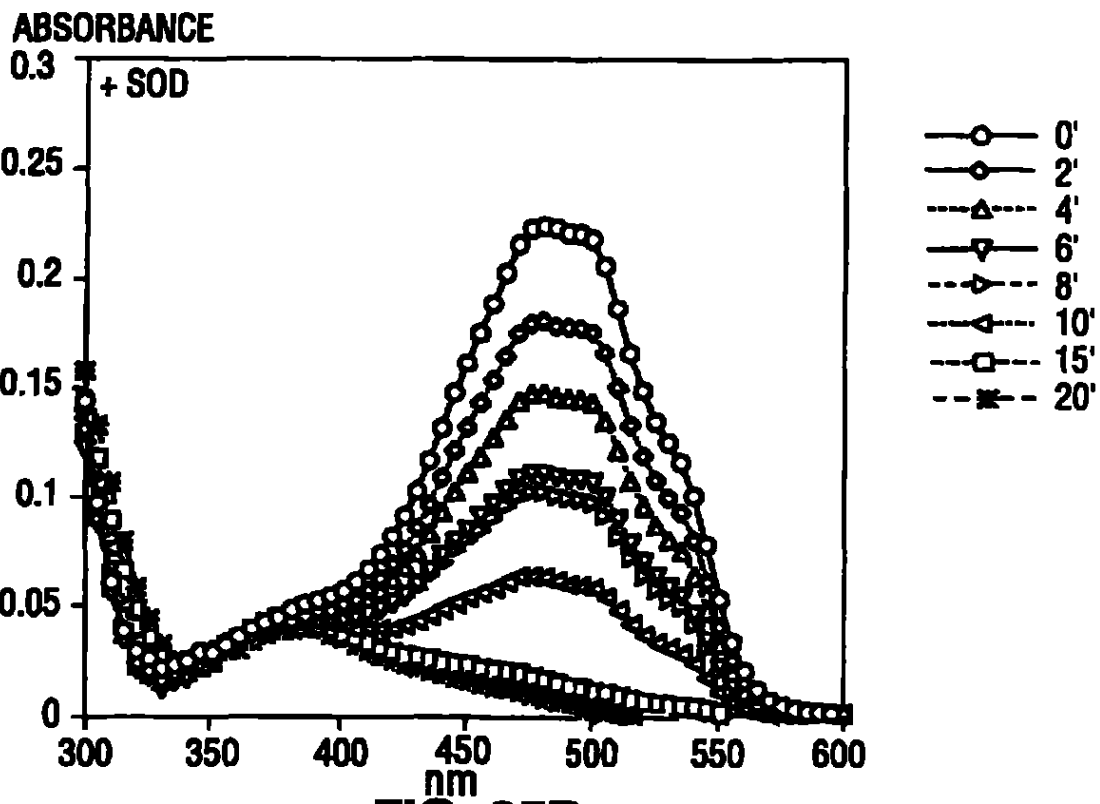


FIG. 25B

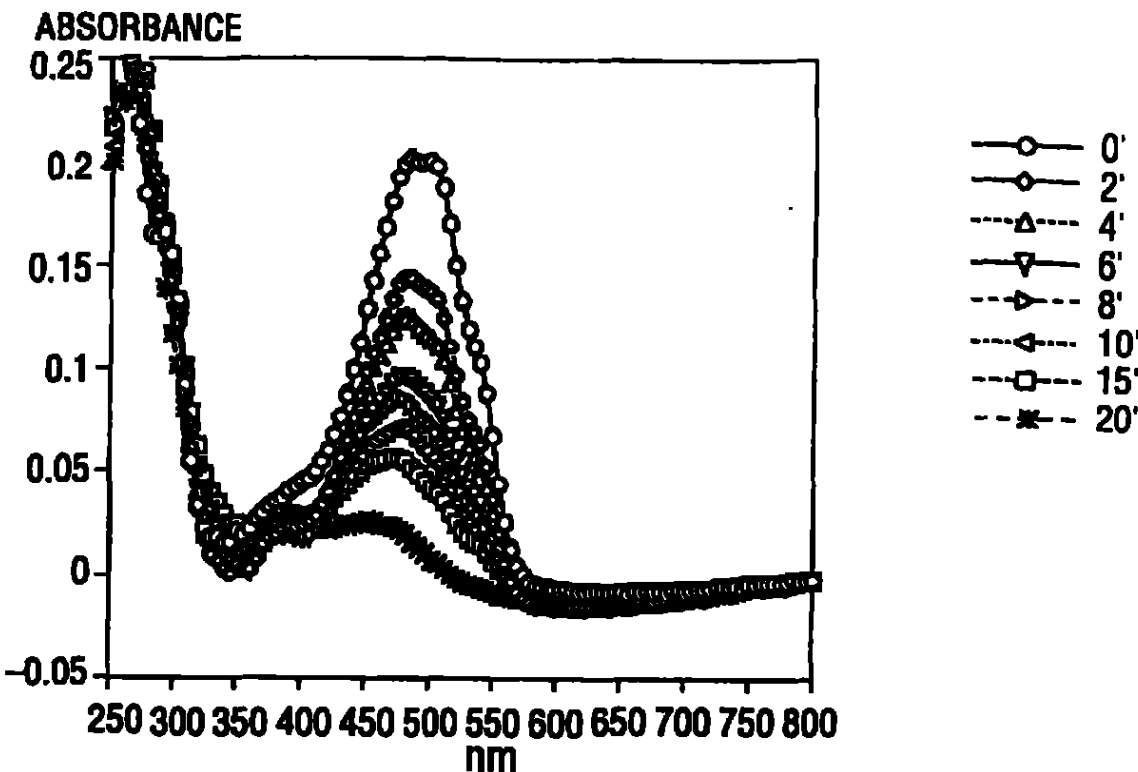


FIG. 26A

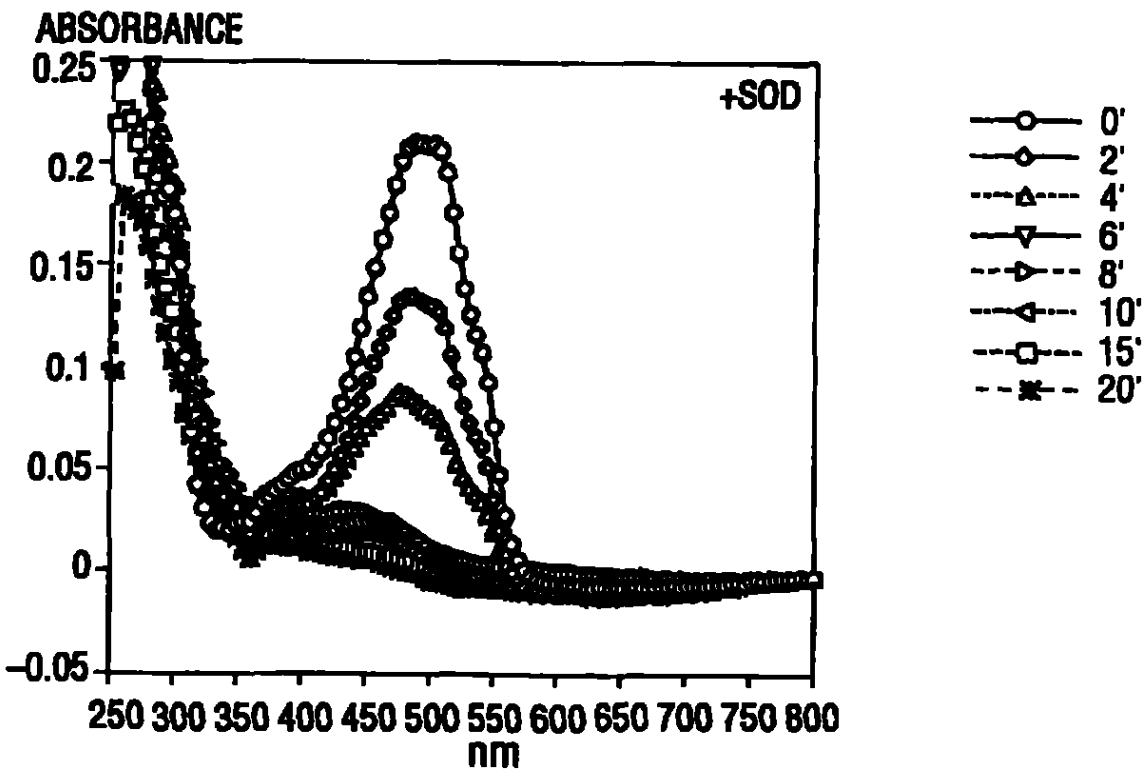


FIG. 26B

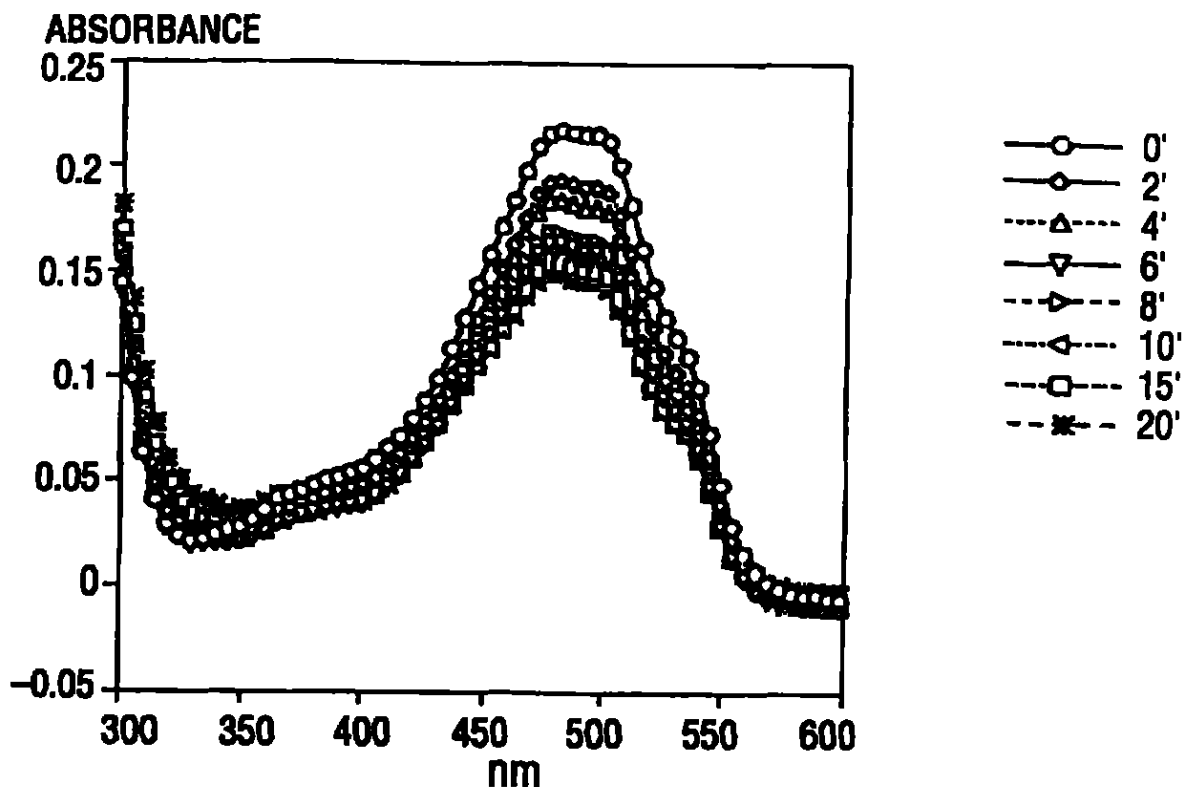


FIG. 27A

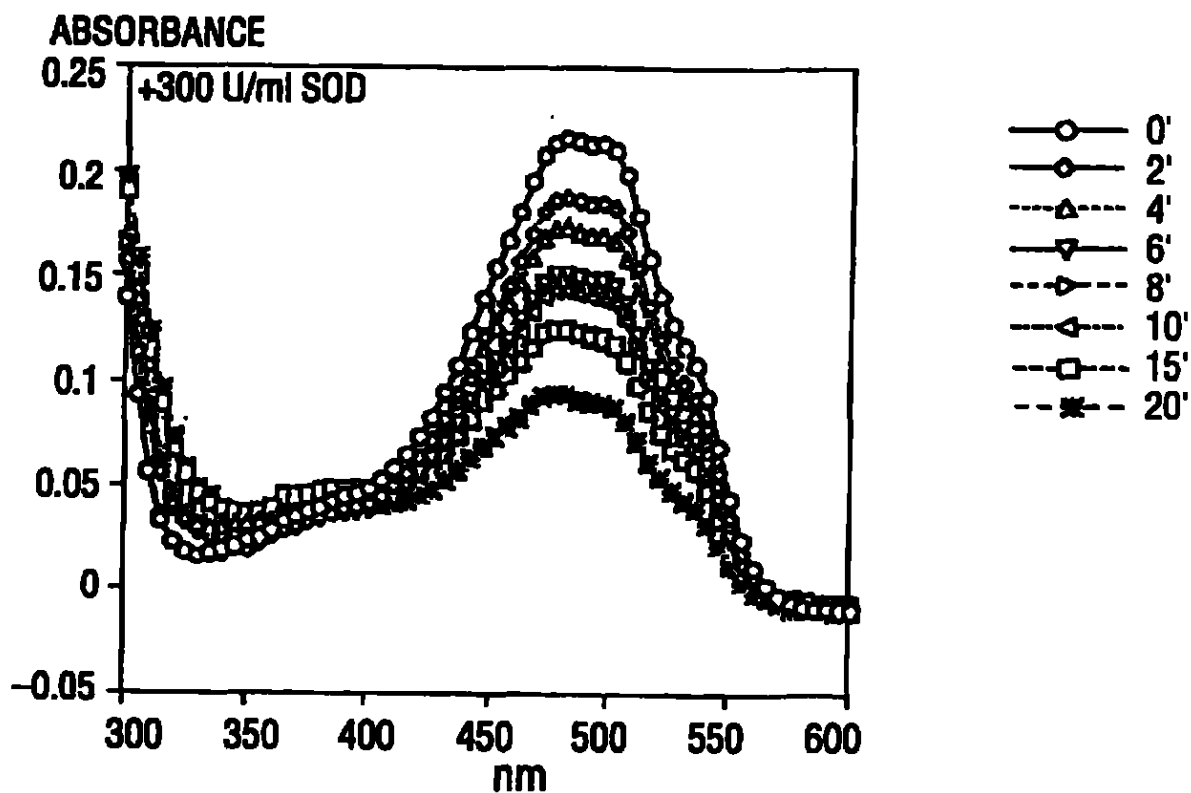


FIG. 27B

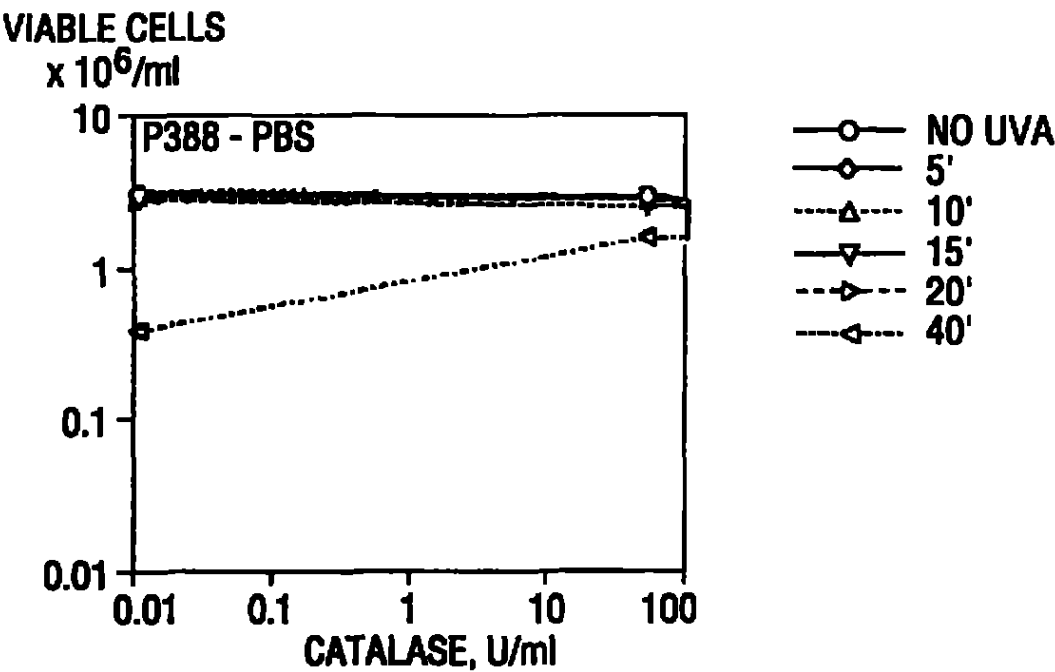


FIG. 28A

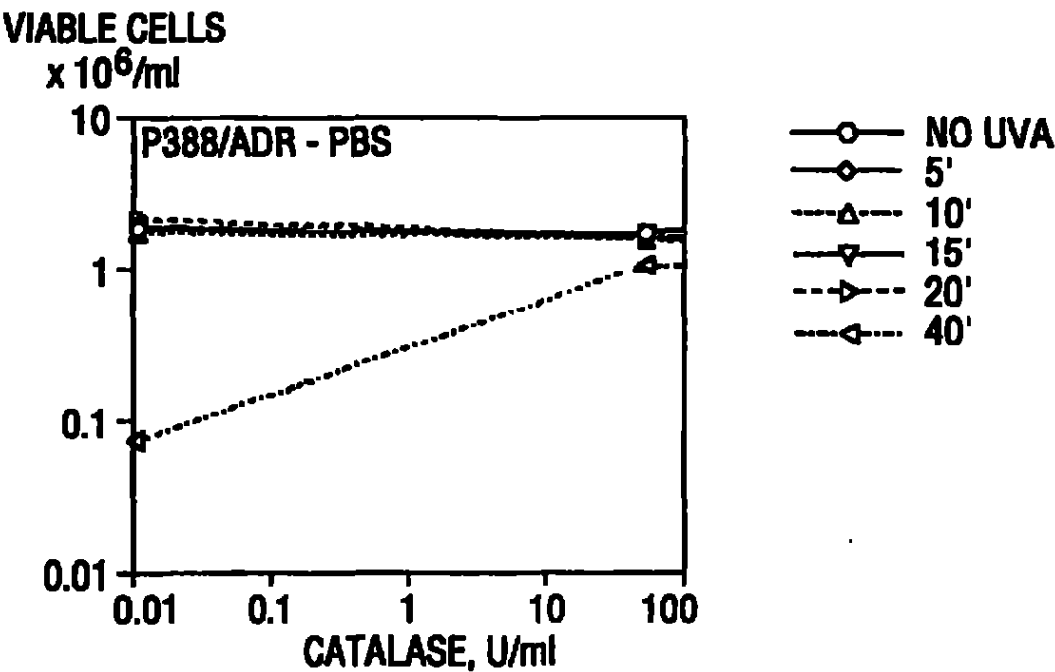


FIG. 28B

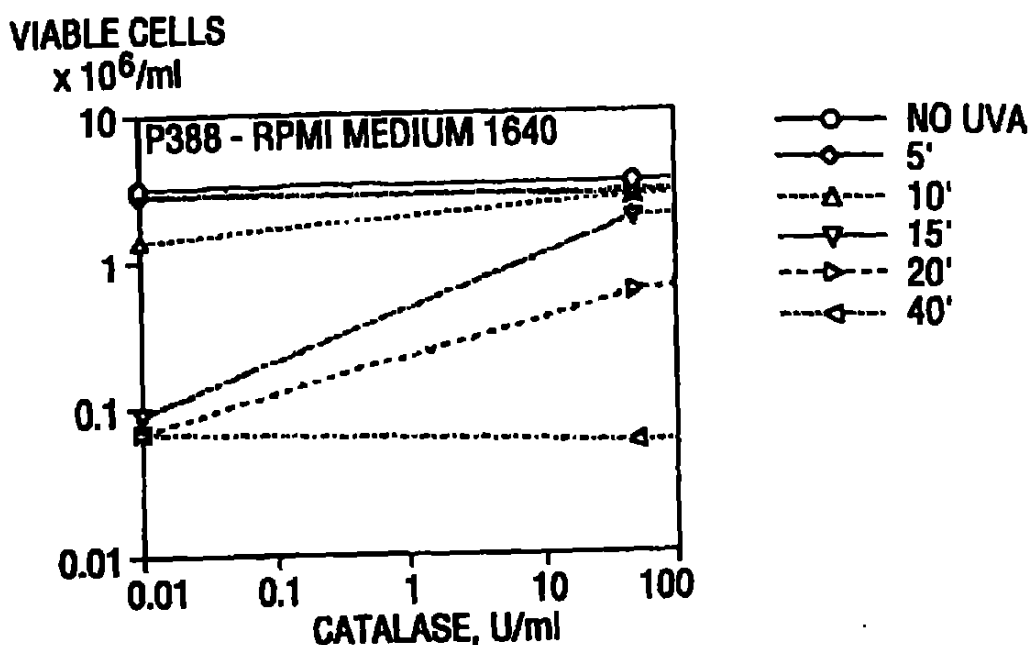


FIG. 28C

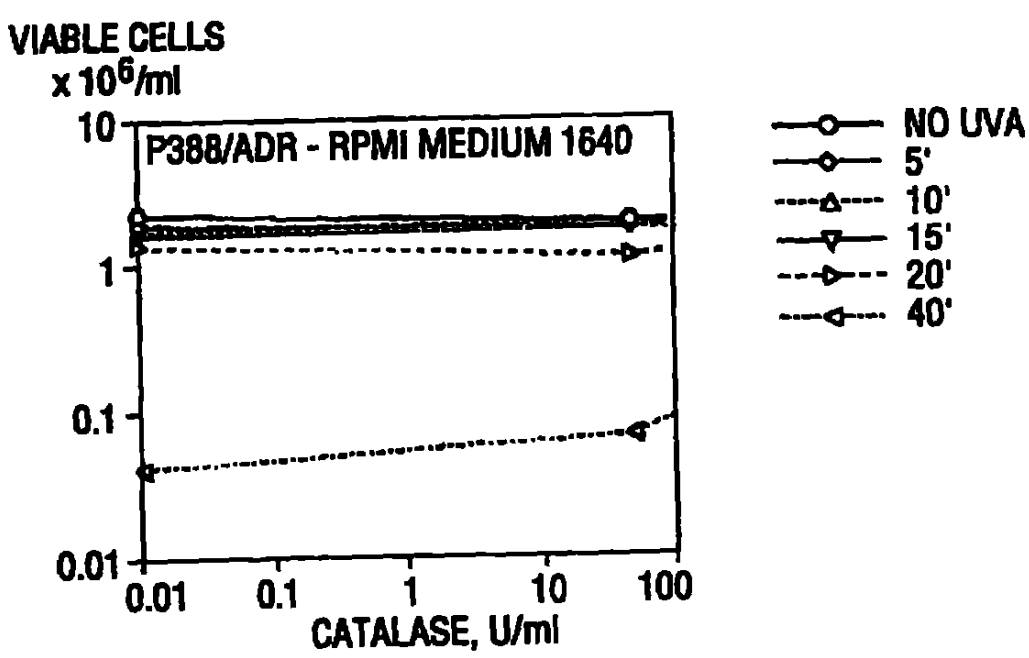


FIG. 28D

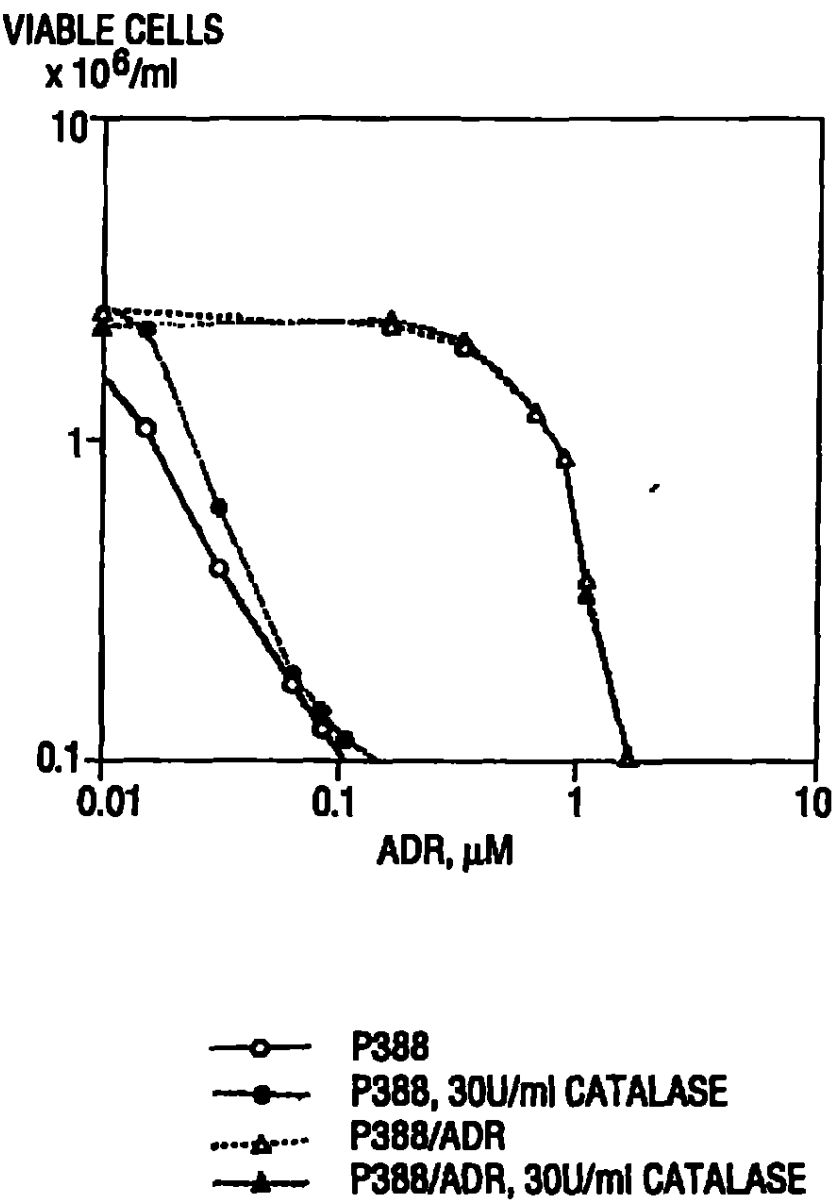


FIG. 29

5.780.446

1

FORMULATIONS OF VESICANT DRUGS AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

The present invention relates to methods of treating and or preventing extravasation injury and more particularly, to photochemotherapeutic methods of prophylaxis and/or treatment of extravasation injury induced by vesicant antineoplastic drugs and pharmaceutical formulations for use therein.

BACKGROUND OF THE INVENTION

Despite the enormous efforts and resources directed at finding a cure, cancer remains an elusive and deadly foe for mankind. The standard methods of treatment usually include chemotherapy, radiation treatment, and surgical removal or tumors and/or growths, or some combination thereof. These treatments, combined with an emphasis on preventative lifestyle modification, have afforded a measure of success in the battle against some cancers. However, cancer remains one of the leading causes of mortality, and cancers detected at matured stages are invariably fatal.

Numerous chemical agents have been devised for the treatment of cancer and they exhibit varying degrees of efficacy. However, no single drug has one hundred percent effectiveness against different cancers, and negative side-effects ranging from minor to serious are always present. Indeed, few medications in common use have a narrower therapeutic index and a greater potential for causing harmful side effects than do antineoplastic drugs.

Most antineoplastic drugs are administered by intravenous injection or infusion. Many of these drugs are vesicants or local irritants and produce severe soft tissue damage upon infiltration or extravasation into tissue surrounding an injection or infusion site. Vesicant compounds include a number of DNA-binding and/or DNA-intercalating antineoplastic agents, examples of which include aclarubicin, actinomycin-D (ACT-D), amosacrine (M-AMSA), carmustine (BCNU), bisantrene, cisplatin, dacarbazine, daunorubicin (DAUNO), doxorubicin (DOX), epirubicin (ESO), estramustine phosphate, epirubicin, estoposide, mechlorethamine, mithramycin (MITH), mitoguanzone, mitomycin-C, mitoxanthrone, streptozotocin, taxol, teniposide, vinblastine, vincristine, vindesine, zinostatin, and zorubicin [Goodman & Gilman's *The Pharmacological Basis of Therapeutics*, 9th ed. (1996) MacGraw Hill, New York]. Of these, the anthracyclines such as doxorubicin (DOX) are particularly well-known clinically for causing severe tissue necrosis upon paravenous extravasation. Doxorubicin-induced ulcers are unique in their prolonged course with slow healing and their propensity to invade deep structures such as tendons and joints without respect to fascial planes. (Rudolph et al. (1976) *Cancer* 38:1087). Thus, extravasation injury associated with vesicant antineoplastic agents and anthracyclines in particular presents a significant and potentially disabling side effect of cancer therapy.

Apart from measures to prevent paravenous extravasation in the first place, there has been little success with therapeutic compounds or methods to treat or prevent extravasation injury. Purported antidotes such as sodium bicarbonate, corticosteroids, propranolol, isoproterenol, N-acetylcysteine, glutathione, lidocaine, bupivacaine, hyaluronidase, diphenhydramine, cimetidine, alpha-tocopherol, dimethyl sulfoxide, and butylated hydroxytoluene have all been shown to be clinically ineffective. Hyper-

2

baric oxygen, topical heat and cold and excision of the infiltrated tissue have also been tried with either unsatisfactory results and/or significant loss of limb function. [Devita et al., *Cancer: Principles and Practice of Oncology*, 3rd edition, Philadelphia, Lippincott, 1989:2386; Dorr (1990) *Blood Rev.* 4:41; Anderson and Dahlstrom (1993), *Eur J Cancer* 29A: 1712]. The management of extravasations resulting from the intravenous administration of antineoplastic agents continues to be controversial.

Therefore, among the many formidable challenges in treating cancer with antineoplastic drugs is the risk of extravasation injury. Clearly, there remains a need for treatment methods or compounds which minimize, prevent, or eliminate the risk of extravasation injury in humans undergoing treatment with antineoplastic agents.

SUMMARY OF THE INVENTION

The present invention relates to methods of treating and or preventing extravasation injury, and more particularly to photochemotherapeutic methods of treatment of extravasation injury induced by vesicant antineoplastic drugs. In accordance with the present invention, extravasation injury is prevented or minimized by the coadministration of photoinactivation inducing compounds (PIC) such as flavins and photoenhancer compounds in a formulation comprising a vesicant antineoplastic and subsequently exposing the injection or infusion site to long ultraviolet irradiation (UVA) or other photoexciting light. While it is not intended that the present invention be limited by the mechanism by which extravasation injury is prevented or treated, it is believed that anthracyclines are photochemically inactivated upon exposure to UVA light when coadministered with photoinactivating inducing compounds (PIC) such as flavins and photoenhancers (e.g., tyrosine, DABCO, HEPES, etc.). Though flavins are known to induce photoinactivation of a number of compounds, the destructive interaction with anthracyclines was unknown. Further, while photochemical reactions between antineoplastics and visible and ultraviolet light have been described, they have not been described in a photosensitizer or photochemotherapy context. [see, e.g., the following references which discuss: methotrexate: Lichtenstien and Goldman (1970) *Biochem. Pharmacol.* 19:1229; Vinca alkaloids: Granzow et al. (1995) *Cancer Res.* 55:4837; mitomycin C: Yajima and Mizunoya (1981) *J. Biochem.* 89:929 and anthracyclines: Sanfilippo et al. (1968) *Glom. Microbiol.* 16:50; Verini et al. (1968) *Glom. Microbiol.* 16:55; Di Marco et al. (1972) *Experientia* 28:327; Daugherty et al. (1979) *Biochem. Biophys. Acta* 565:13; Williams and Tritton (1981) *Photochem. Photobiol.* 34:131; Gray and Phillips (1981) *Photochem. Photobiol.* 33:297; Gray et al. (1982) *Photochem. Photobiol.* 36:49; and Yee et al. (1984) *Cancer Res.* 44:1898]. Therefore, it has been surprisingly found that the use of photosensitizers, in particular flavins, when combined with anthracyclines in a formulation, greatly accelerates the rate of anthracycline inactivation upon exposure to UVA irradiation (i.e., 320-400 nm). Thus, the cytotoxic activity of the anthracyclines is eliminated in localized tissues, thereby eliminating the risk of extravasation injury, while the cytotoxic activity is retained in non-UVA irradiated tissues.

In one embodiment, the present invention provides a method of treatment, comprising: a) providing: i) a subject; and ii) a formulation comprising a photoinactivation inducing compound; and b) administering intravenously said formulation to said subject; and c) exposing the intravenous administration site to UVA light. In a preferred embodiment, the subject is a mammal and in a particularly preferred

5.780.446

3

embodiment, a human. In another preferred embodiment, the formulation further comprises a vesicant chemotherapeutic.

The method of the present invention is not limited by the nature of the chemotherapeutic used. In one embodiment, the chemotherapeutic is an antineoplastic drug. In another embodiment, the antineoplastic drug is an anthracycline or an anthracycline derivative. A particularly preferred anthracycline is doxorubicin. In another embodiment the antineoplastic is a Vinca alkaloid (e.g., vinblastine, vincristine and vindesine).

The present invention is not limited by the photoinactivation inducing compound employed. In one embodiment, the photoinactivation inducing compound is a flavin which is preferably selected from the group consisting of riboflavin, flavin mononucleotide, and flavin adenine dinucleotide.

In a preferred embodiment, the method of the present invention uses a formulation which further comprises at least one photoenhancer compound. The present invention is not limited by the photoenhancer compound chosen. In one embodiment, the photoenhancer compound is a tertiary aliphatic amine, such as 1,4-diazabicyclo(2.2.2)octane. In another embodiment, the photoenhancer compound is a piperazine, such as N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid and 1,4-dimethylpiperazine. In a still further embodiment, the photoenhancer compound is selected from the group consisting of tyrosine, tryptophan, histidine, methionine, superoxide dismutase and ethylenediaminetetraacetic acid (EDTA). In another embodiment, the method of the present invention employs a formulation further comprising catalase.

In one embodiment, the present invention provides a method of preventing or treating extravasation injury in a human undergoing intravenous chemotherapy, comprising: a) providing: i) a human subject; and ii) a formulation comprising a vesicant chemotherapeutic and a photoinactivation inducing compound; and b) administering intravenously said formulation under conditions such that said vesicant chemotherapeutic is not inactivated; and c) exposing the intravenous administration site to UVA light under conditions such that a portion of said vesicant chemotherapeutic is photoinactivated. The irradiation is conducted such that the vesicant chemotherapeutic which has extravasated into the tissues surrounding the injection site is photoinactivated but the vesicant chemotherapeutic present in the vessel or at sites of intended therapy is not significantly photoinactivated.

The method of the present invention is not limited by the nature of the chemotherapeutic used. In one embodiment, the chemotherapeutic is an antineoplastic drug. In another embodiment, the antineoplastic drug is an anthracycline or an anthracycline derivative. A particularly preferred anthracycline is doxorubicin. In another embodiment the antineoplastic is a Vinca alkaloid (e.g., vinblastine, vincristine and vindesine).

The present invention is not limited by the photoinactivation inducing compound employed. In one embodiment, the photoinactivation inducing compound is a flavin which is preferably selected from the group consisting of riboflavin, flavin mononucleotide, and flavin adenine dinucleotide.

In a preferred embodiment, the method of the present invention uses a formulation which further comprises at least one photoenhancer compound. The present invention is not limited by the photoenhancer compound chosen. In one

4

embodiment the photoenhancer compound is a tertiary aliphatic amine, such as 1,4-diazabicyclo(2.2.2)octane. In another embodiment, the photoenhancer compound is a piperazine, such as N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid and 1,4-dimethylpiperazine. In a still further embodiment, the photoenhancer compound is selected from the group consisting of tyrosine, tryptophan, histidine, methionine, superoxide dismutase and ethylenediaminetetraacetic acid (EDTA). In another embodiment, the method of the present invention employs a formulation further comprising catalase.

The present invention further provides a composition comprising a serum-free saline solution comprising a vesicant chemotherapeutic and a photoinactivation inducing compound. In one embodiment, the saline solution is a 0.9% saline solution (i.e., normal saline). In a preferred embodiment, the composition further comprises dextrose (e.g., 5% dextrose). In still another embodiment, the composition further comprises lactose and/or methylparaben. The compositions of the present invention are not limited by the type of chemotherapeutic employed. In one embodiment, the chemotherapeutic is an antineoplastic drug. In another embodiment, the antineoplastic drug is an anthracycline or an anthracycline derivative. A particularly preferred anthracycline is doxorubicin. In another embodiment, the antineoplastic is a Vinca alkaloid.

The present invention is not limited by the photoinactivation inducing compound employed. In one embodiment, the photoinactivation inducing compound is a flavin which is preferably selected from the group consisting of riboflavin, flavin mononucleotide, and flavin adenine dinucleotide.

In a preferred embodiment, the composition of the present invention further comprises at least one photoenhancer compound. The present invention is not limited by the photoenhancer compound chosen. In one embodiment, the photoenhancer compound is a tertiary aliphatic amine, such as 1,4-diazabicyclo(2.2.2)octane. In another embodiment, the photoenhancer compound is a piperazine, such as N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid and 1,4-dimethylpiperazine. In a still further embodiment, the photoenhancer compound is selected from the group consisting of tyrosine, tryptophan, histidine, methionine, superoxide dismutase and EDTA.

In a preferred embodiment, the composition contains the vesicant antineoplastic and the photoinactivation inducing compound in a ratio of between 80:1 and 1:1. In another embodiment, the composition further comprises catalase.

The present invention also provides a composition comprising a vesicant chemotherapeutic, a photoinactivation inducing compound and at least one photoenhancer compound. The composition is preferably serum-free.

The compositions of the present invention are not limited by the type of chemotherapeutic employed. In one embodiment, the chemotherapeutic is an antineoplastic drug. In another embodiment, the antineoplastic drug is an anthracycline or an anthracycline derivative. A particularly preferred anthracycline is doxorubicin. In another embodiment the antineoplastic is a Vinca alkaloid.

The present invention is not limited by the photoinactivation inducing compound employed. In one embodiment, the photoinactivation inducing compound is a flavin which is preferably selected from the group consisting of riboflavin, flavin mononucleotide, and flavin adenine dinucleotide.

In a preferred embodiment, the composition of the present invention further comprises at least one photoenhancer

B

compound. The present invention is not limited by the photoenhancer compound chosen. In one embodiment, the photoenhancer compound is a tertiary aliphatic amine, such as 1,4-diazabicyclo(2.2.2)octane. In another embodiment, the photoenhancer compound is a piperazine, such as N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid and 1,4-dimethylpiperazine. In a still further embodiment, the photoenhancer compound is selected from the group consisting of tyrosine, tryptophan, histidine, methionine, superoxide dismutase and EDTA. In another embodiment, the composition further comprises catalase.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B show the effect of the length of UVA irradiation of DOX dissolved in RPMI medium 1640 or in phosphate buffered saline (PBS), respectively on the growth inhibitory effect of the drug on P388 cells.

FIGS. 2A-2F show the effect of length of UVA irradiation of 20 μ M DOX in various media with 50 mM HEPES (pH=7.2) on the absorbance spectrum of DOX.

FIGS. 3A-3F show the effect of length of UVA irradiation of 20 μ M DOX in various media with 50 mM HEPES (pH=7.2) on the absorbance spectrum of DOX.

FIG. 4 shows the effect of an 8 min UVA irradiation of 0.3 μ M DOX dissolved with FMN or FAD in 0.2 ml PBS on the growth inhibitory effect of DOX on P388 cells that were added after the irradiation in 0.8 ml growth medium.

FIGS. 5A and 5B show the effect of the length of UVA irradiation of 20 μ M DOX dissolved in PBS, in the absence or presence of 20 μ M riboflavin, on the absorbance spectrum of DOX.

FIGS. 6A and 6B show the effect of the length of UVA irradiation of 20 μ M riboflavin dissolved in PBS, in the absence or presence of 20 μ M DOX, on the absorbance spectrum of riboflavin.

FIG. 7 shows the effect of the presence of ascorbic acid on the changes that UVA irradiation induce in the absorbance spectrum of 20 μ M DOX irradiated in RPMI medium 1640 without phenol red.

FIG. 8 shows the effect of the length of UVA irradiation of 0.3 μ M DOX containing increasing amounts of riboflavin in PBS (pH=7.2) on the growth inhibitory effect of DOX on P388 cells.

FIG. 9 shows the effect of an 8 minute UVA irradiation of 0.3 μ M DOX containing increasing amounts of FMN or FAD in PBS (pH=7.2) on the growth inhibitory effect of DOX on P388 cells.

FIGS. 10A-10C show the effect of the length of UVA irradiation of 20 μ M DOX dissolved in PBS, in the absence or presence of 2 or 20 μ M riboflavin, on the absorbance spectrum of DOX.

FIG. 11A shows the effect of 20 minutes of UVA irradiation of 20 μ M DOX with 5 mM DABCO dissolved in PBS and increasing concentrations of riboflavin on the absorbance spectrum of DOX.

FIG. 11B shows the effect of 20 minutes of UVA irradiation of 20 μ M DOX with 2 μ M riboflavin dissolved in PBS and increasing concentrations of DABCO on the absorbance spectrum of DOX.

FIGS. 12A and 12B show the effect of the length of UVA irradiation of 20 μ M DOX with 2 μ M riboflavin in PBS, in the absence or presence of 5 mM DABCO on the absorbance spectrum of DOX.

FIGS. 13A-13C show the effect of the length of UVA irradiation of 20 μ M DOX with 20 μ M riboflavin in PBS, in

the absence or presence of 5 mM DABCO on the absorbance spectrum of DOX.

FIGS. 14A and 14B show the effect of the length of UVA irradiation of 20 μ M DOX with 20 μ M FAD in PBS, in the absence or presence of 5 mM DABCO on the absorbance spectrum of DOX.

FIGS. 15A and 15B show the effect of the length of UVA irradiation of 20 μ M DOX with 20 μ M riboflavin in PBS, in the absence or presence of 5 mM HEPES on the absorbance spectrum of DOX.

FIG. 16 shows the effect of UVA irradiation of 20 μ M DOX with 5 μ M riboflavin, 5 mM HEPES and 0, 20 or 80 μ M tyrosine in 0.1M NaCl on the absorbance spectrum of DOX.

FIG. 17 shows the effect of UVA irradiation of 20 μ M DOX with 40 μ M tyrosine, 5 mM HEPES and 0, 0.25, 0.5 or 1 μ M riboflavin in 0.1M NaCl on the absorbance spectrum of DOX.

FIG. 18 shows the effect of UVA irradiation of 20 μ M DOX with 0.5 μ M riboflavin, 100 μ M tyrosine and 0, 0.5, or 5 mM HEPES in PBS on the absorbance spectrum of DOX.

FIGS. 19A-19C show the effect of 0 to 20 minutes of UVA irradiation of 20 μ M DOX with 0.5 μ M riboflavin and increasing concentrations of tyrosine in PBS (lacking Ca^{2+} and Mg^{2+}) on the absorbance spectrum of DOX.

FIGS. 20A and 20B show the effect of UVA irradiation of 20 μ M DOX with 100 μ M tyrosine and with or without 0.5 μ M riboflavin in PBS on the absorbance spectrum of DOX.

FIGS. 21A and 21B show the effect of UVA irradiation of 20 μ M DOX with 0.5 μ M riboflavin, 100 μ M tyrosine and 0 or 5 mM 1,4-Dimethylpiperazine in PBS on the absorbance spectrum of DOX.

FIG. 22 shows the effect of the length of UVA irradiation of various concentrations of DOX containing 2 μ M riboflavin and 5 mM DABCO in PBS on the growth inhibitory effect of DOX on P388 cells.

FIGS. 23A-23C show the effect of UVA irradiation of 20 μ M DOX with 0.5 μ M riboflavin with or without 100 μ M tryptophan and with or without 5 mM HEPES in PBS on the absorbance spectrum of DOX.

FIG. 24 shows the effect of UVA irradiation of 20 μ M DOX with 0.5 μ M riboflavin with or without 100 μ M phenylalanine, tryptophan, tyrosine, methionine, histidine or EDTA in PBS (without Ca^{2+} and Mg^{2+}) on the absorbance spectrum of DOX.

FIGS. 25A and 25B show the effect of UVA irradiation of 20 μ M DOX with 0.5 μ M riboflavin, 100 μ M tyrosine and with or without 300 U/ml SOD in PBS (without Ca^{2+} and Mg^{2+}) on the absorbance spectrum of DOX.

FIGS. 26A and 26B show the effect of UVA irradiation of 20 μ M DOX with 20 μ M riboflavin and with or without 300 U/ml SOD in PBS (without Ca^{2+} and Mg^{2+}) on the absorbance spectrum of DOX.

FIGS. 27A and 27B show the effect of UVA irradiation of 20 μ M DOX with 2 μ M riboflavin and with or without 300 U/ml SOD in PBS (without Ca^{2+} and Mg^{2+}) on the absorbance spectrum of DOX.

FIGS. 28A-28D show the effect of catalase (21,000 U/ml) on the growth inhibition of P388 cells and P388/ADR cells by UVA irradiation in 0.2 ml of either PBS or RPMI medium 1640.

FIG. 29 shows the effect of 30 U/ml catalase (21,000 U/ml) on the growth inhibitory effect of DOX on P388 cells and P388/ADR cells.

5.780.446

7

DEFINITIONS

To facilitate understanding of the invention, a number of terms are defined below.

The term "chemotherapeutic" refers to a compound (i.e., a drug or agent) administered to an animal, including a human, for the treatment of disease, including but not limited to infectious disease and cancer. Chemotherapeutics encompass antibiotics as well as antineoplastics.

The term "antineoplastic" refers to a compound (i.e., a drug or agent) administered to an animal, including a human, for the treatment of cancers or neoplasms.

The term "vesicant chemotherapeutic" refers to chemotherapeutic agents or drugs which are vesicants or local irritants. Vesicant chemotherapeutics produce soft tissue damage which can be severe upon infiltration or extravasation into tissue surrounding an injection or infusion site. Vesicant chemotherapeutics comprise a number of DNA-binding and/or DNA-intercalating antineoplastic agents [e.g., aciarubicin, actinomycin-D (ACT-D), amsacrine (M-AMSA), BCNU, bisantrene, cisplatin, dacarbazine, daunorubicin (DAUNO), doxorubicin (DOX), esorubicin (ESO), estramustine phosphate, epirubicin, estoposide, meclorothamine, mitramycin (MITH), mitoguazone, mitomycin-C, mitoxanthrone, streptozotocin, taxol, teniposide, vinblastine, vincristine, vindesine, zalcitabine, and zorubicin].

The term "formulation comprising a vesicant chemotherapeutic" refers to a composition comprising a vesicant chemotherapeutic. The formulation may contain other active compounds such as PICs and photoenhancers as well as inactive compounds such as excipients (e.g., diluents, expanders, binders, carriers, preservatives, stabilizing agents, buffers, etc.).

The term "photosensitizer" as used herein refers to a compound which absorbs light energy and passes it to other molecules which themselves cannot absorb energy at the wavelength(s) of light which excite the photosensitizer.

The term "photoinactivation inducing compounds" is not presently used in the art but is introduced here to describe aspects of the present invention. The terms photoinactivation inducing compounds or "PICs" refer to compounds which when present in a solution containing an anthracycline mediate the UVA-inactivation of the anthracycline. Photoinactivation inducing compounds encompass but are not limited to photosensitizers such as flavins.

The term "light-inactivation of an anthracycline" refers to either 1) a reduction in the growth inhibitory activity of the anthracycline or 2) a decrease in the absorbance of an anthracycline solution following exposure to light. The growth inhibitory activity of an anthracycline is demonstrated by growing cells in culture in medium containing or lacking an anthracycline. A reduction in the ability of the cells to grow in medium containing the anthracycline as compared to growth in the absence of the anthracycline demonstrates the growth inhibitory activity of the anthracycline. The growth of culture cells is conveniently measured by counting the number of viable cells per milliliter of culture medium after a given period of incubation (see, e.g., the assay described in Example 1). In addition, as shown herein, a reduction in the growth inhibitory activity of anthracyclines correlates with structural changes in the anthracycline which may be detected by obtaining the absorbance spectrum (between 350 to 600 nm) of the DOX solution (maximum at 480 nm) (e.g., using the assay described in Example 2). A decrease in the absorbance

8

spectrum of the DOX between 425 and 550 nm reflects light-inactivation of the DOX.

The term "photoenhancer" refers to compounds which enhance or accelerate the PIC-mediated photoinactivation of anthracyclines (e.g., DOX). As shown herein, the presence of a photoenhancer (e.g., DABCO, HEPES, tyrosine, tryptophan, etc.) in an anthracycline solution lacking a PIC has little or no effect on the light photoinactivation of the anthracycline. However, when a photoenhancer is added to a solution containing an anthracycline (e.g., DOX) and a PIC (e.g., a flavin), the light-induced photoinactivation of the anthracycline is greatly enhanced. The addition of a photoenhancer to a formulation containing an anthracycline and a PIC allows either 1) a lower dose of light (e.g., a shorter irradiation time) to be used to inactivate the anthracycline, 2) a lower concentration of PIC to be used to achieve the same level of inactivation of the anthracycline (relative to the concentration of PIC required in the absence of a photoenhancer), 3) or both.

The term "flavin" refers to riboflavin and derivative of riboflavin, including but not limited to FMN and FAD.

The term "piperazine" refers to piperazine (i.e., diethylenediamine) and piperazine derivatives (e.g., N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid and 1,4-dimethylpiperazine).

Light plays an important role in many dermatological conditions. This has led to the fields of photobiology, photodiagnosis and phototherapy. Light is one part of the electromagnetic spectrum. The electromagnetic waves from the sun which play a part in photobiological reactions are mainly long ultraviolet light (300-400 nm; UVB and UVA) and visible light (400-760 nm). UV radiation is divided into three wavelength regions, UVC, UVB and UVA, based upon biological-physical factors such as the capacity to induce erythema or melanin pigmentation.

UVA comprises the 320-400 nm wavelength of the electromagnetic spectrum. In small doses, UVA provokes neither erythema nor pigmentation, while in high doses it can cause erythema and immediate pigmentation and in combination with UVB, an intensified erythema. UVA in doses up to 100 J/cm² does not lead to histologically or phototoxic changes in the epidermis nor to marked dilation of the vessels in the upper dermis. Just as in reontegen therapy, defined units exist for UV radiation: Watt-output or intensity of the lamp; Watt-second=joule (J); and Joule=unit of energy. The unit of dosage, i.e., the energy supplied (J) is given per unit surface area (skin surface): W/sec/cm²=J/cm².

"Phototoxicity" is a photochemically evoked inflammatory skin reaction in an irradiated region without an immunological basis. The principle of phototoxicity is used in photochemotherapy or psoralens-UVA (PUVA). While sunburn is caused by a quantitative radiation overdose, the precipitation of a phototoxic reaction requires a photosensitizer in the presence of radiation. Photosensitizers may arise endogenously (e.g., porphyrins) or may be supplied exogenously via the skin, gastrointestinal tract or parenterally (e.g., drugs) and the light can be visible or long ultraviolet.

DESCRIPTION OF THE INVENTION

The present invention relates to improved formulations of vesicant anticancer agents, and more particularly, to novel formulations of anthracyclines especially doxorubicin, such that the side effects of extravasation are reduced or eliminated. The description of the invention is divided into the following sections: (I) Properties of Anthracyclines and

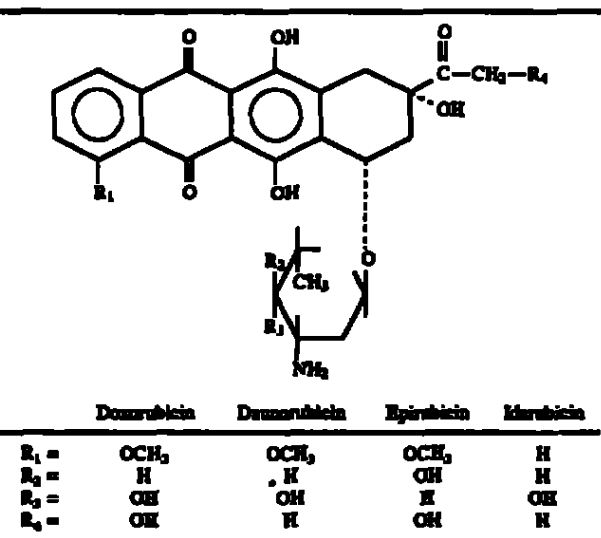
85

Other Vesicant Chemotherapeutic Drugs: (II) Photochemotherapy and Photoinactivation Inducing Compounds; (III) Properties of the Novel Anthracycline Formulations; (IV) Production of Novel Anthracycline Formulations; and (V) Method of Treating or Preventing Extravasation Injury. I. Properties Of Anthracyclines And Other Vesicant Chemotherapeutic Drugs

The anthracyclines are members of the Rhodomycin group of antibiotics produced by *Streptomyces peuceletii* and include doxorubicin, daunorubicin and idarubicin. They have considerable activity against a wide array of tumors, with daunorubicin and idarubicin used primarily in the acute leukemias whereas doxorubicin displays broader activity against human neoplasms, including a variety of solid tumors. The clinical value of these agents is limited by an unusual cardiomyopathy, the occurrence of which is related to the total dose of the drug and is often irreversible.

In a search for agents with high antitumor activity but reduced cardiac toxicity, hundreds of anthracycline derivatives and related compounds have been prepared. Several of these have shown promise in clinical studies, including idarubicin, eprubicin, and the synthetic compound mitoxantrone, which is an amino anthracenedione [Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th ed. (1996) MacGraw Hill, New York].

The anthracycline antibiotics have tetracycline ring structures with an unusual sugar, daunosamine, attached by glycosidic linkage. Cytotoxic agents of this class all have quinone and hydroquinone moieties on adjacent rings that permit them to function as electron-accepting and -donating groups (see following structure).



Daunorubicin, idarubicin, doxorubicin and analogs of doxorubicin (e.g., mitoxantrone) are usually administered intravenously and are rapidly cleared from the plasma. Recommended dosages comprise, for doxorubicin hydrochloride: 60 to 75 mg/m² administered as a single rapid intravenous (IV) injection at 21 day intervals; for daunorubicin hydrochloride: 30 to 60 mg/m² administered IV daily for 3 days; idarubicin hydrochloride: 12 mg/m² administered IV daily for 3 days; and mitoxantrone: 12 mg/m² administered daily for 3 days.

Toxic manifestations of doxorubicin and daunorubicin are similar. Myelosuppression is a major dose-limiting complication. Stomatitis, gastrointestinal disturbances, and alopecia are common but reversible. Erythematous streaking near the site of infusion (i.e., Adriamycin Flare) is a benign local

allergic reaction and is not to be confused with extravasation. The drugs may also produce severe local toxicity in X-ray irradiated tissues (e.g., the skin, heart, lung, esophagus, and gastrointestinal mucosa) which may occur even when the two therapies are not administered concomitantly.

Anthracyclines are also members of a larger class of vesicant anticancer agents which includes intercalating antibiotics such as mithramycin; alkylating agents (mitomycin, dibarbazine); and the Vinca alkaloids (vinblastine, vincristine, and vindesine). All vesicant drugs may induce extravasation injury by infiltrating into surrounding tissue at the infusion site. This is characterized histologically by the presence of ischemic necrosis without inflammation, early vascular obliteration and collagen necrobiosis [Luedke et al. (1979) *Plast. Reconstr. Surg.* 63:463].

II. Photochemotherapy And Photoinactivation Inducing Compounds

A. Background

Many proliferative skin diseases (psoriasis, vitiligo, eczema, mycosis fungoides, etc), cancerous conditions (T cell lymphomas), and autoimmune disorders are being treated by the combined utilization of photosensitizing chemicals [applied topically or taken orally] plus UVA and/or UVB. Terms such as photosensitization, photochemotherapy, photopheresis and PUVA (psoralens ultra violet A radiation) are commonly used to refer to special applications of this method. Clinically useful behavior has been found in such chemical families as heme-derived products, porphyrins, phthalocyanines, and psoralens. The oldest and most established phototherapeutics are the psoralens or linear furocoumarins in which three major commercial pharmaceuticals dominate: 8-methoxypsoralen (methoxsalen or 8-MOP); 4,5',8-trimethylpsoralen (trioxalen or TMP), and 5-methoxypsoralen (5-MOP).

The widely accepted mechanism of action for psoralens is penetration of the target cell's membrane, intercalation into nuclear DNA, and photo-induced bis 2+2 cycloaddition crosslinking of the double helix. Structural and mechanism studies have shown that the 3,4-double bond and the 4',5'-double bond in psoralen form cyclobutane adducts with the double bonds in the pyrimidine bases of DNA. This renders the DNA unable to uncoil and serve as a template for new gene expression. Thus, the target cell is rendered non-viable [Hurst (1989) *Chemical Research in Toxicology*, 2:69; Anderson and Vorhees (1982) *Ann. Rev. Pharmacol. Toxicol.*, 10:177].

However, all of these classes possesses characteristics which makes them less than ideal in the phototherapeutic function: skin staining, suspected mutagenic/carcinogenic properties, poor absorption rates, and systemic toxicity. More importantly, in all of these cases the compounds are not employed to photoinactivate a coadministered pharmaceutical in order to treat or prevent a non-proliferative skin condition such as extravasation but to enhance the therapeutic effect of irradiation of the skin in proliferative skin diseases.

An in vitro screen for phototherapeutics used to treat proliferative skin diseases has been described [e.g., U.S. Pat. No. 5,356,929, the disclosure of which is herein incorporated by reference]. Beneficial phototherapeutics in the treatment of proliferative skin diseases inhibit binding in cells of epithelial origin with epidermal growth factor (EGF). Epidermal growth factor is a low molecular weight polypeptide which binds to cell surface receptors and which is known to be an important regulator of growth in those cells which possess these particular cell surface receptors. Psoriasis, mycosis fungoides, eczema, cancer, and similar

5.780.446

11

proliferative diseases are often characterized by abnormal cell growth regulation which may be related to the action of EGF on the cells in question. Application of PUVA therapy to correct skin disorders, especially psoriasis, is one clinical expression of photochemotherapy. The use of the assay described above was based on the observation that phototherapeutics for proliferative skin diseases are extremely potent inhibitors of binding of epidermal growth factor to cell surface receptors in mammalian cells including humans and that inhibition of this binding arrests the proliferative disorder. In contrast, in the present invention, a class of compounds called photoactivating inducing compounds (PIC) are defined functionally as those compounds which when coadministered with a chemotherapeutic agent such as an antineoplastic, induce a chemical change in the coadministered chemotherapeutic agent upon exposure to light, such that the chemotherapeutic agent loses its cytotoxic effect.

B. Photochemical Reactions

Photochemical reactions between antineoplastic compounds and light (visible and ultraviolet) have been described [methotrexate (Leichtenstein and Goldman, 1970 *Biochem. Pharmacol.*, 19:1229), Vinca alkaloids (Grazzow et al. 1995, *Cancer Res.*, 55:4837), mitomycin C (Yajima and Mizumoya, 1981, *J Biochem.*, 89:929) and anthracycline (Sanfilippo et al., 1968, *Glom. Microbiol.*, 16:50; Verini et al., 1968, *Glom. Microbiol.*, 16:55; Di Marco et al., 1972, *Experientia*, 28:327; Dougherty et al., 1979, *Biochem. Biophys. Acta.*, 565:13; Williams and Tritton, 1981, *Photochem. Photobiol.*, 34:131; Gary and Phillips, 1981, *Photochem. Photobiol.*, 33:297; Gary et al., 1982, *Photochem. Photobiol.*, 36:49; Yee et al., 1984, *Cancer Res.* 44:1898]. The majority of this literature teaches that light enhances the binding of anthracyclines to DNA and membranes; such an enhancement would be beneficial if it occurred at the intended site of treatment (i.e., the tumor) and detrimental if it occurred elsewhere in the body.

Williams and Tritton report that the cytotoxic activity of anthracyclines is reduced *in vitro* as a result of long ultraviolet (UVA) irradiation [(1991) *Photochem. Photobiol.* 34:131]. The half-time for inactivation of doxorubicin or daunomycin by two 15 W tubes emitting 365 nm light was reported to be 9 hours (Williams and Tritton, *supra*). The length of time required to inactivate anthracyclines under the conditions reported by Williams and Tritton is too long to be practical for clinical applications. Furthermore, Williams and Tritton do not suggest that photoactivation may be used therapeutically to prevent the cytotoxic activity of doxorubicin at undesired sites and the use of photosensitizers (e.g., flavins) to enhance photoinactivation of anthracyclines is not described.

The present invention demonstrates that PICs, such as the flavins, greatly accelerate the rate of inactivation of antineoplastic compounds (e.g., anthracyclines) by UVA irradiation in a localized manner when co-administered within a formulation containing the antineoplastic compound. Using the methods and compositions of the present invention, the cytotoxic activity of anthracyclines is eliminated locally, thereby avoiding the risk of extravasation injury, while retaining the desirable systemic cytotoxic activity in non-UVA irradiated tissues.

III. Properties Of The Novel Anthracyclines Formulations

The present invention provides novel formulations *in vivo* administration comprising vesicant chemotherapeutics (e.g., anthracyclines) and PICs. The presence of the PIC in the chemotherapeutic formulation allows the treatment of extravasation injury caused by the vesicant chemotherapeutic. Sites of extravasation are irradiated with long wave-

12

length ultraviolet light (i.e., 320-400 nm). The presence of the PIC in the chemotherapeutic formulation mediates the photoactivation of the vesicant chemotherapeutic in the tissues surrounding the injection site.

The present invention demonstrates that chemotherapeutics (e.g., anthracyclines such as doxorubicin) can be inactivated by exposure to UVA light (i.e., photoactivated) when a PIC is present in solution with the chemotherapeutic. Chemotherapeutic drugs are toxic to cells. Placing cultured cells in a medium containing a chemotherapeutic drug results in a reduced rate of cell growth or an inhibition of cell growth. As shown herein, the growth inhibitory activity of chemotherapeutic drugs, such as anthracyclines, is not appreciably diminished by exposure of the drug to doses of UVA light typically used in a clinical setting. However, exposure of vesicant chemotherapeutics (e.g., anthracyclines such as DOX) to UVA light in the presence of a PIC causes photoactivation of the anthracycline which results in a reduction in the growth inhibitory activity of the chemotherapeutic. A reduction in the growth inhibitory activity of chemotherapeutic drugs, such as anthracyclines, was shown herein to correlate with structural changes in the drug which are reflected in a decrease in the absorbance spectrum of the drug.

As shown herein, the effects of UVA light on the cell growth inhibitory activity of anthracyclines (e.g., DOX) cell growth inhibitory activity and absorbance spectrum are dependent on the composition of the solution. When the drug was dissolved in PBS, its cell growth inhibitory activity could not be reduced by exposing it, in nmol quantities dissolved in a 1 mm thick solution, to 20 joules of UVA energy. However, in RPMI medium 1640 this irradiation energy was sufficient to destroy about 90% of the drug activity. When 60 nmol of DOX dissolved in a 3.75 mm thick layer of RPMI medium 1640 were exposed to 80 joules of UVA, the drug absorbance between 425 and 550 nm was reduced to background level. When DOX was dissolved in a solution that contained only the inorganic salts of RPMI medium 1640 or in PBS, this UVA energy had only a minor effect on the drug absorbance spectrum. To determine which of the 40 components of the RPMI medium 1640 mediate the effect of UVA irradiation on DOX, studies were carried out in solutions that contained only some of the medium components. These studies revealed that the major mediator of the effect of UVA on DOX was riboflavin, a known photosensitizer.

The present invention also demonstrates that other naturally occurring flavins, such as FMN and FAD, also mediate the effect of UVA on DOX. Although FMN was as effective as riboflavin, the FAD concentration had to be raised 5 fold to obtain a comparable effect. The lower efficacy of FAD in mediating the effect of UVA on DOX may be related to its lower ability to associate with DOX than with riboflavin or FMN [Kharasch and Novak (1981) *Arch. Biochem. Biophys.* 212:20].

It is known that flavins, excited by 365 nm light, can undergo photoreduction while oxidizing certain substrates [Byron and Turnbull (1967) *Photochem. Photobiol.* 6:125]. While not limiting the present invention to any particular mechanism, the changes in the cell growth inhibitory activity and in the absorbance spectrum of DOX after UVA irradiation in the presence of flavins may reflect oxidation of the drug. This possibility is supported by the following observations which are described herein. In the presence of ascorbate (without DOX), UVA-irradiated riboflavin lost its 400 to 500 nm absorbance maximum at a faster rate than that measured in the absence of this reducing agent. It is known

77

that riboflavin in its oxidized state has two absorbance maxima at 360 and 445 nm, while its reduced form shows only the first absorbance maximum [Friedrich (1988) in *Vitamins*, Friedrich, ed., W. de Gruyter, Berlin, pp. 403-471]. The increased rate of loss of riboflavin absorbance at 445 nm when irradiated with UVA in the presence of ascorbate indicated that the photo-excited riboflavin is reduced at a faster rate in the presence of oxidizable substrate (ascorbate). In the presence of DOX, UVA-irradiated riboflavin also lost its 400 to 500 nm absorbance maximum at a faster rate than that measured in the absence of DOX. Therefore, the facilitating effect of DOX on the rate of decrease in riboflavin absorbance at 445 nm under UVA could also reflect an increased rate of reduction of the photo-excited riboflavin because of the availability of oxidizable substrate (DOX). In addition, the relative efficacies of riboflavin, FMN and FAD in mediating the effect of UVA on DOX described herein are similar to those reported previously for their relative abilities to photooxidize ascorbate [Kim et al. (1993) *Photochem. Photobiol.* 57:777]. This similarity further supports the idea that DOX is photooxidized by UVA and flavin. The observation that a reducing agent, ascorbic acid (1.6 mM), blocked the changes occurring in the absorbance spectrum of DOX (20 µM) when irradiated in the presence of 20 µM riboflavin could be interpreted as a competition between oxidizable substrates for the limited availability of an oxidizing agent (e.g., the UVA excited riboflavin). Although it is not yet certain whether DOX is indeed oxidized by UVA and riboflavin, it was previously shown that it could undergo oxidation [Chinami et al. (1984) *Biochem. Int.* 8:299]. Alternatively, the effect of UVA and riboflavin on DOX may involve other chemical reactions, e.g., polymerization. DOX polymers were previously obtained, with a very low quantum efficiency, by irradiating DOX with UVA in the absence of a photosensitizer [Williams and Tritton, *supra*].

In the presence of a photosensitizer, the only amino acids that are known to be affected by UVA irradiation at an appreciable rate are: histidine, methionine, tryptophan and tyrosine [Gollnik (1975), *supra*]. The photosensitizing effect of riboflavin was indeed greatly enhanced in the presence of these (and not the other) amino acids in concentration as in RPMI medium 1640. The mechanism by which histidine, methionine, tryptophan and tyrosine enhance the UVA and riboflavin effect on DOX is not known. Light excited riboflavin was shown to interact directly with certain amino acids, such as tyrosine [Silva and Godoy (1994) *Int. J. Vit. Nutr. Res.* 64:253], and it was also reported that hydrogen peroxide could be generated in a mixture of amino acids and riboflavin exposed to light [Brawley et al. (1993) *Clin. Sci.* 85:709]. This raised the possibility that the hydrogen peroxide was involved in the destruction of DOX. However, as described herein, DOX dissolved in a solution of RPMI medium 1640 inorganic salts, vitamins and amino acids was not protected by catalase from the effect of UVA, it seems to be an unlikely possibility. These results are in accordance with a previous study that found that the riboflavin-sensitized photooxidation of ascorbate also did not involve hydrogen peroxide [Kim et al., (1993) *Photochem. Photobiol.* 57:777].

The present invention also provides novel formulations comprising anthracyclines, PICs and photoenhancers. Photoenhancers are defined as compounds which enhance or accelerate the PIC-mediated photoinactivation of anthracyclines (e.g., DOX). As shown herein, the presence of a photoenhancer (e.g., DABCO, HEPES, tyrosine, tryptophan, superoxide dismutase, EDTA, etc.) in an anthra-

cycline solution lacking a PIC has little or no effect on the UVA photoinactivation of the anthracycline. However, when a photoenhancer is added to a solution containing an anthracycline (e.g., DOX) and a PIC (e.g., a flavin)anthracycline is greatly activation of the anthracycline is greatly enhanced. The addition of a photoenhancer to a formulation containing an anthracycline and a PIC allows either 1) a lower dose of UVA (e.g., a shorter irradiation time) to be used to inactivate the anthracycline, 2) a lower concentration of PIC to be used to achieve the same level of inactivation of the anthracycline (relative to the concentration of PIC required in the absence of a photoenhancer), 3) or both.

IV. Production Of Novel Anthracycline Formulations

The present invention demonstrates that anthracyclines (e.g., DOX) in solution can be chemically changed with a concomitant loss of growth inhibitory activity and change in absorbance spectrum by long ultraviolet light UVA at intensities that are used clinically. To obtain this effect, the anthracycline is combined in a formulation with a photoinactivation inducing compounds (PICs) (e.g., flavins such as riboflavin, FMN, FAD). In some embodiments, the anthracycline (e.g., DOX) is combined with a flavin at molar quantity ratios of 1:80 to 1:1 flavin:DOX, with:

- a) 1:50 to 1:500 DOX:DABCO molar ratio (or other tertiary aliphatic amines); or
- b) 1:50 to 1:500 DOX:HEPES molar ratio (or other piperazines); or
- c) 1:1 to 1:6 DOX:tyrosine or tryptophan molar ratio (histidine, methionine, or EDTA may be used in place of tyrosine or tryptophan) and with 1:50 to 1:500 DOX:piperazine.

As it was reported that hydrogen peroxide may be formed when amino acids are exposed to UVA in the presence of photosensitizers [Brawley et al. (1993) *Clin. Sci.* 85:709], catalase is added to the formulation in some embodiments in order to protect the tissue from hydrogen peroxide that may be formed when a solution of flavin and amino acids are exposed to UVA. In addition, oxygen radicals may be by-products of the photoinactivation reaction (i.e., the UVA-mediated destruction of anthracycline in the presence of PICs and/or photoenhancers). Accordingly, superoxide dismutase (SOD) is added to the formulation in some embodiments to protect the UVA irradiated tissue from damage due to the generation of oxygen radicals. In addition to protecting tissue from oxygen radicals, as shown herein, SOD further acts as a photoenhancer and accelerates the UVA-mediated destruction of anthracyclines (Ex. 9, *infra*).

Recombinant human CuZn superoxide dismutase is available from Bio-Technology General Corp. (Iselin, N.J.) and has been approved by the FDA (IND 28,225) for use in clinical trials. Bovine superoxide dismutase has been administered subcutaneously to humans (premature infants) at a dose of 0.26 mg/kg [Rosenfeld et al. (1984) *J Pediatr.* 105:781].

V. Method Of Treating Or Preventing Extravasation Injury

The present invention contemplates using therapeutic formulations of chemotherapeutics, such as anthracyclines, photoinactivation inducing compounds (PICs), photoenhancing compounds and hydrogen peroxide degrading agents (e.g., catalase). It is not intended that the present invention be limited by the particular nature of the therapeutic preparation. For example, such compositions can be provided together with physiologically tolerable liquid, gel or solid carriers, diluents, adjuvants and excipients. In addition, the formulations may be combined other antineoplastic agents, including other vesicant drugs such as the Vinca alkaloids. Formulations for such administrations may

5.780.446

15

comprise an effective amount of a vesicant anticancer drug in combination with an effective amount of PICs and/or photoenhancers in sterile water or physiological saline.

On the other hand, formulations may contain such normally employed additives as binders, fillers, carriers, preservatives, stabilizing agents, emulsifiers, buffers and excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions typically contain 1%-95% of active ingredient, preferably 2%-70%.

The compositions are preferably prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared.

The vesicant drug/photoinactivation inducing compound/photoenhancer combinations of the present invention are often mixed with diluents or excipients which are compatible and physiologically tolerable. Suitable diluents and excipients are, for example, water, saline, dextrose, lactose, glycerol, or the like, and combinations thereof. In addition, if desired the compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH buffering agents.

EXPERIMENTAL

The following examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: DOX [Doxorubicin HCl (Adriamycin RDF)]; EDTA (ethylenediaminetetraacetic acid); PBS (Dulbecco's phosphate buffered saline); HCl (hydrogen chloride); NaCl (sodium chloride); HEPES (N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid]); μ l (microliters); μ g (micrograms); ml (milliliters); L (liters); mg (milligrams); g (grams); hr (hours); mM (millimolar); μ M (micromolar); nM (nanomolar); N (normal); nm (nanometers); min (minutes); sec (seconds); IU (international units); s.c. (subcutaneous); mm (millimeter); MTD (maximally tolerated dosage); I.p. (intraperitoneal); kg (kilograms); UV (ultraviolet); UVA (long ultraviolet light); VIS (visible); W (watt); GibcoBRL (GibcoBRL, Gaithersburg, Md.); Sigma (Sigma Chemical Co., St. Louis, Mo.).

Riboflavin, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), ascorbic acid and catalase (2200 units/mg protein) were purchased from Sigma. RPMI medium 1640, RPMI medium 1640 without riboflavin, RPMI medium 1640 without phenol red, Dulbecco's phosphate-buffered saline (PBS) and HEPES buffer solution were purchased from GibcoBRL. RPMI medium 1640 Select-Amine Kit, which contained: a) 4x concentrate of inorganic salt mixture ($\text{Ca}^{2+}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 400 mg/L, KCl 1600 mg/L, MgSO_4 195.36 mg/L, NaCl 24000 mg/L); b) 100x concentrate of vitamin mixture (Biotin 20 mg/L, D- Ca^{2+} pantothenate 25 mg/L, choline-Cl 300 mg/L, folic acid 100 mg/L, i-inositol 3500 mg/L, niacinamide 100 mg/L, p-aminobenzoic acid 100 mg/L, pyridoxine.HCl 100 mg/L, riboflavin 20 mg/L, thiamine.HCl 100 mg/L, vitamin B₁₂ 0.5 mg/L and reduced glutathione 100 mg/L); and c) 100x concentrate of amino acid mixture (l-arginine 20000 mg/L, l-asparagine 5000 mg/L, l-aspartic acid 2000 mg/L, l-cystine 5000 mg/L, l-glutamic acid 2000 mg/L, l-glutamine 30000 mg/L, glycine 1000 mg/L, l-histidine 1500 mg/L, l-hydroxyproline 2000 mg/L, l-isoleucine 5000 mg/L, l-leucine 5000 mg/L, l-lysine.HCl 4000 mg/L, l-methionine 1500 mg/L, l-phenylalanine 1500 mg/L,

16

l-proline 2000 mg/L, l-serine 3000 mg/L, l-threonine 2000 mg/L, l-tryptophan 500 mg/L, l-tyrosine 2000 mg/L and l-valine 2000 mg/L.) was purchased from GibcoBRL. Doxorubicin HCl (Adriamycin RDF) was purchased from Farmitalia Carlo Erba (Milan, Italy).

EXAMPLE 1

In Vitro Cell Culture Assay For Monitoring The Photoinactivation Of Anthracyclines

An in vitro assay was employed which allows for the rapid screening of various formulations for their ability to enhance UVA-mediated inactivation of anthracyclines. Application of solutions containing anthracyclines to the culture medium of cells causes an inhibition of the growth of the cells (i.e., the anthracyclines are cytotoxic). The growth inhibitory activity of anthracyclines was found to be reduced by exposure of the anthracycline solution to long ultraviolet light (UVA) prior to the addition of cultured cells. The reduction in growth inhibitory activity was found to correlate with a decrease in the absorbance spectrum of the anthracycline (e.g., doxorubicin) (described in Example 2). This in vitro assay permits the rapid screening of compounds for the ability to enhance the photoinactivation of anthracyclines by UVA irradiation. The in vitro assay is illustrated below using the P388 cell line; however, any mammalian cell line can be employed.

a) Cell Culture

Cell culture was carried out as previously described [Ramu et al. (1984) *Cancer Res.* 44:144]. Briefly, P388 murine leukemia cells (i.e., P388D₁, available from the ATCC; ATCC CCL 46) were maintained in RPMI medium 1640 supplemented with 10% fetal calf serum (FCS), penicillin base (50 units/ml) and streptomycin (50 μ g/ml) (all from GibcoBRL) and 10 μ M 2-mercaptoethanol (Sigma) (herein after "complete culture medium"). An inoculum of cells was transferred to fresh medium once every 4 days to maintain exponential growth. Four days after the seeding of 1×10^5 viable cells/ml, cell growth was assessed by measurements of cell density in a Coulter Counter with multi-sizer (Coulter Electronics Ltd., Beda, England). Sensitivity to DOX was assessed by culturing the cells in 24 well cell culture clusters (Costar, Cambridge, Mass.) in a volume of 1 ml/well with various drug concentrations and by measuring cell density 4 days later.

b) Long Ultraviolet (UVA) Light Exposure

Various concentrations of DOX were dissolved in RPMI medium 1640 or other solutions described infra (referred to as drug solutions or DOX solutions). Drug solutions (0.2 ml/well) in 24 well culture clusters without the lid were irradiated in a laminar flow hood with 3 Blacklight Blue 40 W lamps (Vilber Lourmat, Marne la Vallee, France). The energy flow rate delivered to the DOX solutions was 5 to 6 mW/cm² as measured with a Cole-Parmer 97503-00 Radiometer (Niles, Ill.) with a 365 nm sensor. The hood air flow was found to be sufficient to prevent warming of the irradiated solutions throughout the length of the experiments. After the exposure of the DOX solutions to UVA, 1×10^5 viable cells in 0.8 ml of the complete culture medium were added to each well, and the cell density was measured after 4 days of culture.

FIG. 1 shows the effect of the length of UVA irradiation of DOX, dissolved in RPMI medium 1640 or in PBS, on the growth inhibitory effect of the drug on P388 cells that were added after the irradiation of the drug solution. For the results shown in FIG. 1A, various concentrations of DOX (0.01, 0.1, 1.0, 10 or 100 μ M), dissolved in 0.2 ml of RPMI

5.780.446

17

medium 1640, were placed in the wells of a 24 well culture plate and the plate was exposed to UVA for 0, 5, 10, 20 or 30 minutes as described above. P388 cells were then added to each well (1×10^5 viable cells in 0.8 ml of complete culture medium) and the plates were incubated for 4 days. Following the incubation, the density of the cells in each well was determined.

The results shown in FIG. 1B were obtained as described for FIG. 1A with the exception that the DOX was dissolved in PBS rather than RPMI medium 1640 prior to irradiation.

The results shown in FIG. 1 demonstrated that irradiating DOX in PBS for up to 30 minutes did not affect the growth inhibitory effect of the drug, but irradiating DOX in RPMI medium 1640 resulted in a decrease of the drug activity that was proportional to the length of irradiation. When RPMI medium 1640 was irradiated prior to adding the drug, no reduction in the growth inhibitory effect was observed: this suggested that if the UVA activated some component(s) of the RPMI medium 1640 that could inactivate the drug, this component decayed rapidly and did not affect the DOX that was added later. Replacing the standard RPMI medium 1640 with RPMI medium 1640 without phenol red did not affect the results, indicating that the dye did not participate in the drug inactivation process.

EXAMPLE 2

Effect of Long Ultraviolet Light Irradiation On The Absorbance Spectrum of Doxorubicin

The results shown above demonstrated that UVA irradiation ameliorated the growth inhibitory activity of DOX. In order to investigate whether this photoinactivation reflected structural changes in the DOX molecule, the following experiments were conducted.

a) Spectral Measurements

Three milliliters of 20 μ M DOX solutions were placed in 35 mm open cell culture dishes and irradiated with UVA in a laminar flow hood for up to 20 min. (as described above). After the irradiation, the 350 to 600 nm absorbance spectra were measured in a double beam UV-VIS scanning spectrometer (Shimadzu Scientific Instruments Inc., Columbia, Md.). In some experiments, to deplete the dissolved oxygen, the drug solutions were bubbled with nitrogen for 10 min prior to and through the length of exposure to UVA. The DOX absorbance (measured at 480 nm) decayed exponentially over the length of the UVA irradiation time. The first order rate constant of the decrease in DOX absorbance (K_{DOX}) was calculated. In repeated experiments, the standard deviation of this parameter was consistently <17% of the mean value.

b) Exposure To Long Ultraviolet Light Effects The Absorbance Spectrum Of Doxorubicin

Because the reduction in the DOX growth inhibitory activity by UVA irradiation may reflect structural changes in the DOX molecule, the effect of UVA irradiation on the absorbance spectrum of DOX was studied. The results are summarized in FIGS. 2 and 3.

In FIGS. 2 and 3, various solutions containing 20 μ M DOX and 50 mM HEPES (pH 7.2) was irradiated for 0, 2, 4, 6, 8, 10, 15 or 20 minutes and the absorbance between 350-600 nm was measured. FIG. 2A shows the absorbance spectra obtained when DOX was dissolved in RPMI medium 1640 without phenol red. FIG. 2B shows the absorbance spectra obtained when DOX was dissolved in RPMI medium 1640 without riboflavin. FIG. 2C shows the absorbance spectra obtained when DOX was dissolved in a solution containing the inorganic salts found in RPMI

18

medium 1640. FIG. 2D shows the absorbance spectra obtained when DOX was dissolved in a solution containing the 20 amino acids and inorganic salts found in RPMI medium 1640. FIG. 2E shows the absorbance spectra obtained when DOX was dissolved in a solution containing the vitamins and inorganic salts found in RPMI medium 1640. FIG. 2F shows the absorbance spectra obtained when DOX was dissolved in a solution containing the vitamins, 20 amino acids and inorganic salts found in RPMI medium 1640.

FIG. 3A shows the absorbance spectra obtained when DOX was dissolved in a solution containing the inorganic salts, vitamins and histidine, methionine, tryptophan and tyrosine found in RPMI medium 1640. FIG. 3B shows the absorbance spectra obtained when DOX was dissolved in a solution containing the inorganic salts, vitamins and all amino acids found in RPMI medium 1640 except histidine, methionine, tryptophan and tyrosine. FIG. 3C shows the absorbance spectra obtained when DOX was dissolved in a solution containing the inorganic salts and riboflavin found in RPMI medium 1640. FIG. 3D shows the absorbance spectra obtained when DOX was dissolved in a solution containing the inorganic salts, riboflavin and histidine, methionine, tryptophan and tyrosine found in RPMI medium 1640. FIG. 3E shows the absorbance spectra obtained when DOX was dissolved in a solution containing the inorganic salts and vitamins except riboflavin found in RPMI medium 1640. FIG. 3F shows the absorbance spectra obtained when DOX was dissolved in a solution containing the inorganic salts, vitamins except riboflavin and histidine, methionine, tryptophan and tyrosine found in RPMI medium 1640.

The results shown in FIG. 2 demonstrate that irradiating the drug in RPMI medium 1640 without phenol red containing 50 mM HEPES (to maintain pH=7.2) resulted in a decrease in absorbance in the 425-550 nm range. This decrease was an exponential function of the irradiation time with a first order rate constant of 0.025/min. When DOX was dissolved in a solution containing only the inorganic salts of RPMI medium 1640, the effect of UVA irradiation on the absorbance spectrum of DOX was 10 fold slower ($K_{DOX}=0.002/\text{min}$).

Adding the 20 amino acids that are present in RPMI medium 1640 to its inorganic salts in concentration as in this medium accelerated somewhat the decrease in DOX absorbance resulting from the UVA irradiation ($K_{DOX}=0.004/\text{min}$). Adding the vitamin mixture to the inorganic salt solution also accelerated this reaction ($K_{DOX}=0.007/\text{min}$). However, when the amino acid and vitamin mixtures were added together to the inorganic salt solution, the reaction rate ($K_{DOX}=0.029/\text{min}$) reached a level comparable to that obtained in the standard RPMI medium 1640.

As shown in FIG. 3, to maintain the reaction at a high rate ($K_{DOX}=0.025/\text{min}$), it was sufficient to add just the following 4 amino acids: histidine, methionine, tryptophan and tyrosine with the vitamins. Furthermore, the other 16 amino acids did not accelerate the effect of UVA and vitamins on the absorbance spectrum of DOX ($K_{DOX}=0.003/\text{min}$). Without the vitamin mixture, histidine, methionine, tryptophan and tyrosine did not enhance the UVA irradiation effect on DOX ($K_{DOX}=0.002/\text{min}$). These results indicated that the effect of UVA on DOX in RPMI medium 1640 is carried out through some component(s) of the vitamin mixture serving as a photosensitizer and that the photosensitizer effect is enhanced by at least one of the following amino acids: histidine, methionine, tryptophan and tyrosine.

Riboflavin is a known photosensitizer [Girotti (1983) Photochem. Photobiol. 38:745]. Adding riboflavin to the

concentration found in RPMI medium 1640 (0.5 μ M) to the inorganic salt mixture somewhat accelerated the reaction ($K_{DOX}=0.006/\text{min}$). However, if histidine, methionine, tryptophan and tyrosine were also added, the reaction was highly accelerated ($K_{DOX}=0.022/\text{min}$). The effect of UVA irradiation on DOX dissolved in RPMI medium 1640 without riboflavin (FIG. 2) proceeded at a rate ($K_{DOX}=0.007/\text{min}$) that was significantly slower than that observed in the standard RPMI 1640. Similarly, the addition of a vitamin mixture lacking riboflavin to the inorganic salt mixture (FIG. 3) resulted in marginal acceleration of the reaction ($K_{DOX}=0.004/\text{min}$), which was not enhanced by adding histidine, methionine, tryptophan and tyrosine ($K_{DOX}=0.005/\text{min}$).

These data indicated that the major component mediating the destruction of DOX dissolved in RPMI medium 1640 by UVA is riboflavin and that this effect is accelerated, i.e., enhanced, by the presence of one or more of the following amino acids: histidine, methionine, tryptophan and tyrosine. In addition, the data suggested that the amino acid and vitamin mixtures may contain other minor photosensitizing components.

These results also demonstrate that the amelioration of the DOX growth inhibitory activity by UVA irradiation (shown in Example 1) correlates with structural changes in the DOX molecule. Therefore, the ability of a compound, alone or in combination with other compounds, to decrease the absorbance of DOX between 425 and 550 nm following UVA irradiation may be used to rapidly screen compounds for the ability to photoinactivate DOX.

EXAMPLE 3

The Effect Of Long Ultraviolet Light On Doxorubicin Is Mediated By Flavins

The preceding example demonstrated that the major component mediating the destruction of DOX dissolved in RPMI medium 1640 by UVA is riboflavin. Although many compounds have been shown to be modified by UVA and flavins (Giroli, supra), DOX has not previously been identified as such a compound. In this example, the ability of various flavins to cause the photoinactivation of DOX was examined by cell culture assays and spectral studies.

a) Effect Of Flavins And UVA Upon The Growth Inhibitory Effect Of DOX

DOX, at a concentration of 0.3 μ M, was dissolved in 0.2 ml PBS with increasing concentrations of FMN or FAD. These solutions were then irradiated with UVA for 8 min. After irradiation, 1×10^5 viable P388 cells in 0.8 ml complete growth medium were added to each well. After 4 days of culture, the cell density was measured. The results are presented in FIG. 4.

In FIG. 4, the number of viable cells ($\times 10^4/\text{ml}$) is plotted against the concentration of flavin (FMN or FAD) present in the DOX solution during irradiation. In FIG. 4, the results obtained using a solution containing either FMN or FAD in the absence of DOX and without irradiation are shown by the solid lines containing either an open circle or a filled circle ($\circ$, $\bullet$), respectively. The dotted lines containing either an open or a filled diamond ($\diamond$, $\blacklozenge$) represent the results obtained from irradiating a solution containing either FMN or FAD in the absence of DOX, respectively. The dashed lines containing either an open or a filled triangle (Δ , $\blacktriangle$) represent the results obtained using a solution containing either FMN or FAD in the presence of DOX but without irradiation, respectively. The dashed lines containing either open or filled inverted triangles (∇ , $\blacktriangledown$) represent the results obtained using a solution containing either FMN or FAD in the presence of DOX and irradiation, respectively.

As can be seen in FIG. 4, UVA irradiation of DOX in PBS for 8 minutes did not affect the growth inhibitory effect of DOX. Similarly, DOX in combination with flavins at the specified concentrations without irradiation, showed no effect on the growth inhibitory effect of DOX. However, UVA irradiation of DOX in the presence of flavins at the specified concentrations obliterated the growth inhibitory effect of DOX on P388 cells. FMN was found to be as effective as riboflavin, and 5 fold more effective than FAD.

b) Effect Of Flavins And UVA Upon The Absorbance Spectrum Of DOX

The effect of UVA irradiation of 20 μ M DOX, 20 μ M riboflavin or 20 μ M DOX with 20 μ M riboflavin in PBS on the absorbance spectra of DOX and riboflavin was measured using the assay described in Example 2. The results are shown in FIGS. 5 and 6.

In FIGS. 5A and 5B, solutions containing 20 μ M DOX in PBS were irradiated for 0, 2, 4, 6, 8, 10, 15 or 20 minutes in the absence or presence of 20 μ M riboflavin, respectively and the absorbance spectrum between 350-600 nm was measured.

In FIGS. 6A and 6B, solutions containing 20 μ M riboflavin in PBS were irradiated for 0, 2, 4, 6, 8, 10, 15 or 20 minutes in the absence or presence of 20 μ M DOX, respectively and the absorbance spectrum between 350-600 nm was measured.

The results shown in FIGS. 5 and 6 demonstrate that in the presence of riboflavin, the UVA irradiation caused a much larger decrease in the absorbance of DOX than in its absence. In the presence of DOX, UVA irradiation of riboflavin resulted in a larger decrease in the vitamin absorbance maximum at 445 nm (but not at 360 nm maximum) than in its absence. These results indicate that the UVA effects on DOX and on riboflavin are enhanced by their combined presence.

EXAMPLE 4

Effects Of Ascorbic Acid, De-aeration And Catalase Upon The Absorbance Spectrum Of DOX

The results shown in Example 3 demonstrated that the effect of UVA irradiation on DOX and on riboflavin were enhanced by the combination of these two compounds. It is known that flavins, excited by 365 nm light, can undergo photoreduction while oxidizing certain substrates [Byron and Turnbull (1967) *Photochem. Photobiol.* 6:125]. Therefore, the changes in the cell growth inhibitory activity and in the absorbance spectrum of DOX after UVA irradiation in the presence of flavins could reflect oxidation of the drug.

To test this hypothesis, the experiment described in Example 3b was repeated as described with the exception that 0.16 or 1.6 mM of ascorbic acid was added to each solution. The absorbance spectrum of DOX irradiated in the presence of riboflavin did not decline, while the absorbance spectrum of riboflavin declined at a much faster rate than that observed in the absence of ascorbate. Thus, ascorbate protected DOX from UVA while enhancing the effect of the irradiation on riboflavin.

The protective effect of ascorbate on DOX from UVA is further demonstrated by the results shown in FIG. 7. FIG. 7 depicts the absorbance spectrum obtained from solutions of 20 μ M DOX in RPMI medium 1640 containing 0, 0.16 or 1.6 mM ascorbate were irradiated for 0, 10 or 20 minutes.

In FIG. 7, the circles represent solutions lacking ascorbate; the open circles represent no irradiation; the hatched

and filled circles represent 10 and 20 minutes of irradiation, respectively. The diamonds represent solutions containing 0.16 mM ascorbate; the open diamonds represent no irradiation; the hatched and filled diamonds represent 10 and 20 minutes of irradiation, respectively. The squares represent solutions containing 1.6 mM ascorbate; the open squares represent no irradiation; the hatched and filled squares represent 10 and 20 minutes of irradiation, respectively.

The results shown in FIG. 7 demonstrate the protective effect of ascorbate. The irradiation of 20 μ M DOX in RPMI medium 1640 (no ascorbate) resulted in decreased DOX absorbance. However, in the presence of 0.16 mM ascorbate this decrease was smaller and in the presence of 1.6 mM ascorbate, even after 20 minutes of irradiation, there were no changes in the absorbance spectrum of DOX.

As demonstrated in Example 3b, UVA irradiation of a solution of 20 μ M DOX and 20 μ M riboflavin in PBS resulted in the decline of DOX absorbance spectrum. Because some of the photosensitizing effects might be mediated through dissolved oxygen, the experiment was repeated after depleting the solution of oxygen by nitrogen bubbling prior to the irradiation. The results indicated that oxygen deprivation had no effect on the decline in DOX absorbance and suggested that oxygen does not participate in the destruction of DOX by the combination of UVA and riboflavin.

Hydrogen peroxide is also known to be produced and participate in certain photosensitizing reactions [Brawley et al. (1993) *Clin. Sci.* 85:709]. Therefore, the effect of adding 150 units/ml catalase (which at room temperature could decompose 450 μ mole/min hydrogen peroxide), to 20 μ M DOX dissolved in RPMI medium 1640 was tested. It was found that the presence of catalase in this solution did not affect the rate of destruction of DOX by the UVA irradiation. This result suggested that it is unlikely that hydrogen peroxide mediates the destruction of DOX by the UVA-riboflavin combination.

EXAMPLE 5

Effect Of Irradiation Time Upon The Growth Inhibitory Activity Of Doxorubicin Solutions Containing Flavins

The effect of increasing the length of UVA irradiation of solutions containing 0.3 μ M DOX in PBS with increasing concentrations of flavins (riboflavin, FMN or FAD) was examined.

The effect of increasing the length of UVA irradiation of solutions containing 0.3 μ M DOX in PBS with increasing concentrations of riboflavin was examined as follows. Two-tenths of a milliliter of 0.3 μ M DOX with increasing concentrations of riboflavin (0 to 15 μ M) in PBS (pH 7.2) was placed in 24 well culture clusters and exposed to 3 Blacklight Blue 40 W lamps (emitting 5-6 mW/cm² at 365 nm) for various amounts of time (0 to 8 min.). Growth medium (0.8 ml) containing 1×10^5 P388 murine leukemia cells was then added and the cultures were allowed to incubate for 4 days and the cell density was determined. The results are shown in FIG. 8.

In FIG. 8, the filled circles and filled triangles represent the density of cells present 4 days after exposure of cells to 0.2 ml of PBS containing increasing amounts of riboflavin (0 to 15 μ M) which received either 0 or 8 minutes of irradiation, respectively (positive controls). The density of cells present after exposure to 0.2 ml of a solution containing 0.3 μ M DOX with increasing concentrations of riboflavin (0

to 15 μ M) in PBS (pH 7.2) and 0, 2, 4, 6, and 8 minutes of irradiation are shown by the open circles, open diamonds, open triangles, open inverted triangles and open side-ways triangles, respectively. It is noted that the final (i.e., after the addition of the cells) concentration of riboflavin present in each well is 5-fold lower than the value shown along the x-axis of FIG. 8 (as well, the final concentration of DOX is 5 fold lower than the stated initial concentration).

The results shown in FIG. 8 demonstrate that the presence of increasing amounts of riboflavin increases the photoinactivation of DOX. As the amount of riboflavin was increased, a gradual loss of the DOX growth inhibitory activity toward 1×10^5 P388 murine leukemia cells was observed. In the presence of 15 μ M riboflavin, the growth inhibitory effect of DOX was destroyed after 8 minutes of exposure to UVA light. Similar effects were obtained in the presence of the riboflavin analogs, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (FIG. 9).

The results shown in FIG. 9 were generated as described above with the exception that FMN or FAD was used in place of riboflavin and the concentrations of flavins varied from 0 to 100 μ M. In FIG. 9, the open and filled symbols represent results obtained using FMN and FAD, respectively. The circles represent results obtained using flavin solutions lacking DOX which were not irradiated. The diamonds represent the results obtained using flavin solutions lacking DOX which were irradiated for 8 minutes. The triangles represent the results obtained using flavin solutions containing DOX which were not irradiated. The inverted triangles represent flavin solutions containing DOX which were irradiated for 8 minutes.

The absorbance spectrum of solutions containing 0.3 μ M DOX in PBS and 0, 2 or 20 μ M riboflavin was determined as described in Example 2; irradiation times varied from 0 to 20 minutes. The results are shown in FIG. 10. FIG. 10A shows absorbance spectrum from solutions containing 0.3 μ M DOX in PBS without riboflavin; FIGS. 10B and 10C show absorbance spectrum from solutions containing 0.3 μ M DOX in PBS with either 2 or 20 μ M riboflavin, respectively. The following symbols indicate 0, 2, 4, 6, 8, 10, 15 or 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, $*$.

The results shown in FIG. 10 demonstrate that the reduction in the growth inhibitory activity of DOX by UVA irradiation resulted from changes in its chemical structure. FIG. 10, shows that riboflavin, in a concentration dependent manner, enhances the decrease in 400 to 550 nm absorbance of DOX which is caused by UVA induced chemical changes.

EXAMPLE 6

The Photoinactivation Of Doxorubicin In The Presence Of Flavins Is Enhanced By Other Compounds

In this experiment, the ability of compounds to enhance the photoinactivation of DOX by flavins was examined. The following compounds were examined: 1,4-diazabicyclo(2,2,2)octane (DABCO), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 1,4-Dimethylpiperazine and tyrosine.

a) The Addition Of DABCO Accelerates The Destruction Of DOX By Riboflavin

The UVA mediated destruction of DOX with riboflavin is enhanced by the presence of 1,4-diazabicyclo(2,2,2)octane (DABCO) (FIGS. 11-13). In FIG. 11A, the effect on the absorbance spectrum of DOX is shown when solutions

containing 5 mM DABCO and 20 μ M DOX in PBS (pH 7.2) and increasing concentrations of riboflavin (0 to 20 μ M) were irradiated for 20 minutes. In FIG. 11A, the following symbols represent 0, 0.5, 1, 2, 5, 10, 15 and 20 μ M riboflavin, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$.

In FIG. 11B, the effect on the absorbance spectrum of DOX is shown when solutions containing 2 μ M riboflavin and 20 μ M DOX in PBS (pH 7.2) and increasing concentrations of DABCO (0 to 50 mM) were irradiated for 20 minutes. In FIG. 11B, the following symbols represent 0, 0.1, 0.5, 1, 2, 5, 10, 20 and 50 mM DABCO, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, $*$, and $\dagger$.

In FIG. 12, the effect of UVA irradiation of solutions containing 20 μ M DOX and 2 μ M riboflavin in PBS (pH 7.2) with or without 5 mM DABCO is shown. In FIG. 12A, the effect on the absorbance spectrum of DOX is shown when solutions containing 2 μ M riboflavin and 20 μ M DOX in PBS (pH 7.2) were exposed to UVA for increasing periods of time. In FIG. 12A, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$. FIG. 12B shows the effect on the absorbance spectrum of DOX is shown when solutions containing 2 μ M riboflavin, 5 mM DABCO and 20 μ M DOX in PBS (pH 7.2) were exposed to UVA for increasing periods of time. In FIG. 12B, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$.

In FIG. 13, the effect of UVA irradiation of solutions containing 20 μ M DOX and 20 μ M riboflavin in PBS (pH 7.2) with or without 5 mM DABCO is shown. In FIG. 13A, the effect on the absorbance spectrum of DOX is shown when solutions containing 20 μ M riboflavin and 20 μ M DOX in PBS (pH 7.2) were exposed to UVA for increasing periods of time. In FIG. 13A, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$. FIG. 13B shows the effect on the absorbance spectrum of DOX when solutions containing 20 μ M riboflavin, 5 mM DABCO and 20 μ M DOX in PBS (pH 7.2) were exposed to UVA for increasing periods of time. In FIG. 13B, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$. FIG. 13C shows the effect on the absorbance spectrum of DOX when solutions containing 20 μ M riboflavin, 5 mM DABCO and 20 μ M DOX in PBS without Ca^{2+} and Mg^{2+} (pH 7.2) were exposed to UVA for very short periods of time. In FIG. 13C, the following symbols represent 0, 15, 30, 45, 60, 75, 90, 105 and 120 seconds of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, $*$, and $\dagger$.

The results shown in FIGS. 11-13 demonstrate that the addition of DABCO further accelerates the destruction of DOX by riboflavin and UVA. In the presence of 5 mM DABCO, 2 μ M riboflavin was sufficient to destroy the DOX absorbance spectrum (20 μ M DOX) in the 425-550 nm range after 20 minutes of UVA irradiation (FIG. 11A) (loss of absorbance in the 425-550 nm range was shown above to correlate with a loss of cytotoxic activity). FIG. 11B shows that in the presence of 2 μ M riboflavin, less than 5 mM, but more than 2 mM, DABCO were sufficient to destroy the DOX absorbance spectrum in the 425-550 nm range after 20 minutes of UVA irradiation. In the presence of 2 μ M riboflavin and 5 mM DABCO, 20 minutes of UVA irradiation were required to destroy the DOX absorbance spectrum (FIG. 12). However, raising the concentration of riboflavin to 20 μ M, decreased the amount of UVA irradiation required to destroy DOX absorbance (FIGS. 13A-C). FIG. 13B shows that less than 2 minutes of UVA irradiation was

required to eliminate the DOX absorbance spectrum at these concentrations and FIG. 13C shows that the DOX absorbance spectrum disappeared in less than 1 minute in the presence of 20 μ M riboflavin and 5 mM DABCO.

b) The Addition Of DABCO Accelerates The Destruction Of DOX By FAD

The UVA mediated destruction of DOX with FAD is enhanced by the presence of 1,4-diazabicyclo(2.2.2)octane (DABCO).

In FIG. 14, the effect of UVA irradiation of solutions containing 20 μ M DOX and 20 μ M FAD in PBS lacking Ca^{2+} and Mg^{2+} (pH 7.2) with or without 5 mM DABCO is shown. In FIG. 14A, the effect on the absorbance spectrum of DOX is shown when solutions containing 20 μ M FAD and 20 μ M DOX in PBS lacking Ca^{2+} and Mg^{2+} (pH 7.2) were exposed to UVA for increasing periods of time. In FIG. 14A, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$.

FIG. 14B shows the effect on the absorbance spectrum of DOX is shown when solutions containing 20 μ M FAD, 5 mM DABCO and 20 μ M DOX in PBS lacking Ca^{2+} and Mg^{2+} (pH 7.2) were exposed to UVA for increasing periods of time. In FIG. 14B, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$.

The results shown in FIG. 14 demonstrate that the addition of DABCO further accelerates the destruction of DOX by FAD and UVA. A comparison of the results shown in FIG. 12 and FIG. 14 demonstrates that FAD was not as effective as riboflavin in mediating the UVA inactivation of DOX, in the presence or absence of DABCO. However, the results shown in FIG. 14 demonstrate that DABCO considerably enhanced the effect of FAD-UVA on DOX.

c) The Addition of Piperazine Compounds And/Or Tyrosine Accelerates The Destruction of DOX By Riboflavin

The ability of piperazine compounds such as N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) and 1,4-Dimethylpiperazine to enhance the riboflavin-mediated UVA destruction of DOX was examined. In addition, the ability of HEPES and 1,4-Dimethylpiperazine to enhance the destruction of DOX by the combination of UVA-riboflavin-tyrosine was examined. Furthermore, the ability of tyrosine to act as a photoenhancer in the absence of piperazine compounds was examined. The UVA irradiation and spectral measurements were carried as described in Example 2.

In FIG. 15, the effect of UVA irradiation of solutions containing 20 μ M DOX and 20 μ M riboflavin in PBS (pH 7.2) in the presence or absence of 5 mM HEPES on the absorbance spectrum of DOX is shown. FIGS. 15A and 15B show the spectrum obtained from solutions containing 20 μ M DOX and 20 μ M riboflavin in PBS (pH 7.2) with and without 5 mM HEPES, respectively. In FIG. 15, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$.

In FIG. 16, the effect of UVA irradiation of solutions containing 20 μ M DOX, 0.5 μ M riboflavin and 5 mM HEPES with 0, 20 or 80 μ M tyrosine in 0.1M NaCl on the absorbance spectrum of DOX is shown. In FIG. 16, the open symbols represent solutions which were not irradiated and the filled symbols represent solutions which received 20 minutes of UVA irradiation. The circles, triangles and inverted triangles represent solutions containing 0, 20 and 80 μ M tyrosine, respectively.

In FIG. 17, the effect of UVA irradiation of solutions containing 20 μ M DOX, 40 μ M tyrosine and 5 mM HEPES with 0, 0.25, 0.5 or 1 μ M riboflavin in 0.1M NaCl on the

absorbance spectrum of DOX is shown. In FIG. 17, the open symbols represent solutions which were not irradiated and the filled symbols represent solutions which received 20 minutes of UVA irradiation. The circles, triangles, inverted triangles and side-ways triangles (◊) represent solutions containing 0, 0.25, 0.5 and 1 μM riboflavin, respectively.

In FIG. 18, the effect of UVA irradiation of solutions containing 20 μM DOX, 0.5 μM riboflavin and 100 μM tyrosine with 0, 0.5, or 5 mM HEPES tyrosine in PBS (pH 7.2) on the absorbance spectrum of DOX is shown. In FIG. 18, the open symbols represent solutions which were not irradiated and the filled symbols represent solutions which received 20 minutes of UVA irradiation. The circles, triangles and inverted triangles represent solutions containing 0, 0.5 and 5 mM HEPES, respectively.

The results shown in FIG. 18 demonstrate that in the presence of 100 μM tyrosine 0.5 μM riboflavin with 20 minutes of UVA irradiation caused a decrease in the absorbance of a 20 μM DOX solution (even in the absence of HEPES). In FIGS. 19A-C, the decrease in the absorbance of DOX exposed to UVA in the presence of 0.5 μM riboflavin was shown to be directly proportional to the concentration of tyrosine. In FIGS. 19A-C, the following symbols represent solutions containing 0, 20, 50, 100, 200, 500 and 1000 μM tyrosine, respectively: ◊, ◊, Δ, ▽, ▷, ◁, and □. FIGS. 19A-C show the absorbance spectrum obtained when a 20 μM DOX solution containing 0.5 μM riboflavin and 0 to 1000 μM tyrosine was either not exposed to UVA irradiation (19A) or exposed to UVA irradiation for 10 (19B) or 20 minutes (19C).

The results shown in FIGS. 20A-B demonstrate that irradiation of a 20 μM DOX solution (in PBS, pH 7.2) containing 100 μM tyrosine has essentially no impact on the absorbance spectrum of DOX but that the addition of 0.5 μM riboflavin to the solution of DOX and tyrosine causes a decrease in the absorbance spectrum of DOX. In FIGS. 20A and B, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: ◊, ◊, Δ, ▽, ▷, ◁, □, and *. FIG. 20A shows the absorbance spectra obtained by irradiation of a 20 μM DOX solution (in PBS, pH 7.2) containing 100 μM tyrosine. FIG. 20B shows the absorbance spectra obtained by irradiation of a 20 μM DOX solution (in PBS, pH 7.2) containing 100 μM tyrosine and 0.5 μM riboflavin.

In FIG. 21, the effect of UVA irradiation of solutions containing 20 μM DOX, 0.5 μM riboflavin and 100 μM tyrosine in PBS (pH 7.2) in the presence or absence of 5 mM 1,4-Dimethylpiperazine on the absorbance spectrum of DOX is shown. FIGS. 21A and 21B show the spectrum obtained from solutions containing 20 μM DOX, 0.5 μM riboflavin and 100 μM tyrosine in PBS (pH 7.2) with and without 5 mM 1,4-Dimethylpiperazine, respectively. In FIG. 21, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: ◊, ◊, Δ, ▽, ▷, ◁, □, and *.

The results shown in FIGS. 15-21 demonstrate that piperazine compounds, such as HEPES, accelerate the riboflavin-mediated photoinactivation of DOX as does DABCO (section a above). However, unlike DABCO, HEPES and other piperazines also enhance the destruction of DOX by the combination of UVA-riboflavin-tyrosine. As shown in FIG. 16, tyrosine (20 to 80 μM) allowed the concentration of riboflavin to be reduced to 0.5 μM (1/40 of the DOX concentration) in the HEPES-containing solution. The results shown in FIG. 17 demonstrate that in the presence of 40 μM tyrosine and 5 mM HEPES, the concentration of riboflavin needed to mediate the destruction of

DOX by UVA light was considerably lower than that required for solutions lacking tyrosine and HEPES (FIG. 10). The results shown in FIG. 18 demonstrate that in the presence of 0.5 μM riboflavin and 100 μM tyrosine, much shorter UVA exposure times were required to destroy DOX in the presence of 5 mM HEPES as compared to solutions containing 0.5 mM or no HEPES. In FIG. 19, it was shown that the decrease in absorbance of DOX exposed to UVA in the presence of 0.5 μM riboflavin and tyrosine is directly proportional to the concentration of tyrosine present in the solution. The results shown in FIG. 21 demonstrate that the enhancement of the destruction of DOX by UVA-riboflavin-tyrosine was also achieved using piperazines other than HEPES.

EXAMPLE 7

The Presence Of DABCO Greatly Accelerates The UVA-Riboflavin-Mediated Inactivation Of Doxorubicin

The benefit of the formulation of DOX with riboflavin and DABCO for the treatment of drug extravasation (with UVA irradiation) is demonstrated in the following *in vitro* experiments. In a previous experiment it was found that irradiation periods longer than 10 minutes were required to prevent the growth inhibitory effects of DOX solutions upon P388 cells when the cell suspensions contained 0 to 3 μM DOX and 2 μM riboflavin. The period of irradiation required to reduce or prevent the cytotoxicity of DOX to P388 cells was next examined using solutions containing DOX and 2 μM Riboflavin and 5 mM DABCO as follows.

Using the cell culture assay described in Example 1, P388 cells were exposed to solutions containing 0 to 3 μM DOX, 2 μM riboflavin and 5 mM DABCO in PBS (pH 7.2) and were irradiated for 0 to 90 seconds. The results are shown in FIG. 22.

In FIG. 22, the following symbols represent the density of cells present 4 days after exposure of cells to UVA in the above solutions for 0, 15, 30, 45, 60 and 90 seconds, respectively: ◊, ◊, Δ, ▽, ▷, and ◁. It is noted that the final concentration (i.e., after the addition of the cells) of DOX present in each well is 5-fold lower than the value shown along the x-axis of FIG. 22.

In experiments described above, it was shown that without UVA irradiation, the presence of 2 μM riboflavin and 5 mM DABCO does not reduce the cytotoxicity of DOX to P388 cells. However, the cytotoxicity of the drug was considerably lowered even after 15 seconds of UVA irradiation and after 1.5 minutes of irradiation about 90% of the drug toxicity was abolished (FIG. 22). This experiment shows that the destruction of DOX cytotoxicity by UVA-riboflavin-DABCO can be achieved without causing damage to the cells that are present throughout the UVA exposure.

EXAMPLE 8

Tryptophan, Methionine, Histidine And EDTA Enhance The Photoinactivation Of Doxorubicin

The preceding examples demonstrated that the addition of tyrosine to formulations of DOX and riboflavin accelerated the UVA-riboflavin-mediated destruction of DOX (i.e., tyrosine acts as a photoenhancer). In this example, the ability of tryptophan, methionine, histidine and EDTA to enhance the photoinactivation of DOX was demonstrated. a) Tryptophan Enhances The Photoinactivation Of Doxorubicin

The absorbance spectrum of solutions containing 20 μ M DOX, 0.5 μ M riboflavin in PBS with and without 100 μ M tryptophan and with or without 5 mM HEPES was determined as described in Example 2. The results are shown in FIG. 23. In FIG. 23, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$. FIG. 23A shows the spectrum obtained from solutions containing 20 μ M DOX and 0.5 μ M riboflavin in PBS. FIG. 23B shows the spectrum obtained from solutions containing 20 μ M DOX and 0.5 μ M riboflavin in PBS with 100 μ M tryptophan. FIG. 23C shows the spectrum obtained from solutions containing 20 μ M DOX and 0.5 μ M riboflavin in PBS with 100 μ M tryptophan and 5 mM HEPES.

The results shown in FIG. 23 shows that the destruction of DOX in a riboflavin formulation by UVA irradiation was enhanced in the presence of tryptophan (FIG. 23B). This effect was further accelerated by adding HEPES (FIG. 23C). b) Methionine, Histidine And EDTA Also Enhance The Photoinactivation Of Doxorubicin

The absorbance spectrum of solutions containing 20 μ M DOX, 0.5 μ M riboflavin in PBS (without Ca^{2+} and Mg^{2+} , pH 7.2) with and without 100 μ M phenylalanine, tryptophan, tyrosine, methionine, histidine or EDTA was determined as described in Example 2. The results are shown in FIG. 24 where the absorbance at 480 nm is plotted against the time of UVA irradiation (0 to 20 minutes). In FIG. 24, the following symbols represent solutions containing only riboflavin, 100 μ M phenylalanine, 100 μ M tryptophan, 100 μ M tyrosine, 100 μ M tyrosine, 100 μ M methionine, 100 μ M histidine, and 100 μ M EDTA, respectively: $\bullet$, $\diamond$, Δ , $\blacktriangle$, ∇ , $\triangleright$, $\triangleleft$ and $*$.

The results shown in FIG. 24 demonstrate that the destruction of DOX in a riboflavin formulation by UVA irradiation was enhanced in the presence of tyrosine, tryptophan, histidine, EDTA, and methionine. From the results shown in FIG. 24, it was determined that tyrosine had the greatest photoenhancing effect followed by tryptophan, histidine, EDTA, and methionine. Phenylalanine did not enhance the riboflavin-mediated destruction of DOX to a significant degree.

EXAMPLE 9

Superoxide Dismutase Enhances The Photoinactivation Of Doxorubicin

As it has been reported that oxygen radicals may be formed when amino acids are exposed to UVA in the presence of photosensitizers, such as flavins, the effect of adding superoxide dismutase (SOD) to formulations of DOX-riboflavin with or without tyrosine upon the UVA-mediated destruction of DOX was examined.

The absorbance spectrum of solutions containing 20 μ M DOX, 0.5 μ M riboflavin, 100 μ M tyrosine in PBS (without Ca^{2+} and Mg^{2+} , pH 7.2) with and without 300 U/ml SOD was determined as described in Example 2. The results are shown in FIGS. 25A and 25B. In FIG. 25, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$. FIG. 25A shows the spectrum obtained from solutions containing 20 μ M DOX, 0.5 μ M riboflavin and 100 μ M tyrosine in PBS. FIG. 25B shows the spectrum obtained from solutions containing 20 μ M DOX, 0.5 μ M riboflavin and 100 μ M tyrosine in PBS with 300 U/ml SOD.

The absorbance spectrum of solutions containing 20 μ M DOX and 20 μ M riboflavin in PBS (without Ca^{2+} and Mg^{2+} , pH 7.2) with and without 300 U/ml SOD was determined as

described in Example 2. The results are shown in FIGS. 26A and 26B. In FIG. 26, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$. FIG. 26A shows the spectrum obtained from solutions containing 20 μ M DOX and 20 μ M riboflavin in PBS. FIG. 26B shows the spectrum obtained from solutions containing 20 μ M DOX and 20 μ M riboflavin in PBS with 300 U/ml SOD.

The absorbance spectrum of solutions containing 20 μ M DOX and 2 μ M riboflavin (i.e., 10-fold less than that used in FIG. 26) in PBS (without Ca^{2+} and Mg^{2+} , pH 7.2) with and without 300 U/ml SOD was next determined as described in Example 2. The results are shown in FIGS. 27A and 27B. In FIG. 27, the following symbols represent 0, 2, 4, 6, 8, 10, 15 and 20 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, $\triangleleft$, $\square$, and $*$. FIG. 27A shows the spectrum obtained from solutions containing 20 μ M DOX and 2 μ M riboflavin in PBS. FIG. 27B shows the spectrum obtained from solutions containing 20 μ M DOX and 2 μ M riboflavin in PBS with 300 U/ml SOD.

The results shown in FIGS. 25-27 demonstrate that superoxide dismutase acts as a photoenhancer and accelerates the rate at which DOX is inactivated by either riboflavin or riboflavin-tyrosine. Because oxygen radicals may be generated during the photoinactivation of DOX formulations containing flavins and amino acid enhancers (e.g., tyrosine), the addition of SOD to the DOX formulation serves the dual purpose of protecting the UVA irradiated tissue from free radicals as well as enhancing the destruction of DOX.

EXAMPLE 10

Catalase Protects Cells From The Effects of UVA Irradiation

It has been reported that hydrogen peroxide may be formed when amino acids are exposed to UVA in the presence of photosensitizers, such as flavins (Brawley et al., supra). Therefore, the addition of agents capable of degrading hydrogen peroxide to DOX formulations containing flavins and/or amino acids would serve to protect tissue exposed to UVA from damage due to the production of hydrogen peroxide. In this example the ability of catalase to protect cells from damage caused by protracted UVA irradiation was examined.

P388 cells and P388/ADR cells in either PBS or RPMI 1640 medium containing 0.01 to 100 U/ml catalase were exposed to UVA light for increasing periods of time as described in Example 1b with the exception that 1×10^6 cells were placed in the 0.2 ml solution (PBS or RPMI with catalase) prior to irradiation. After irradiation, 0.8 ml complete culture medium was added to each well and the cell density was measured after 4 days of culture. The P388/ADR cell line is a subclone of the P388 cell line which was selected for multidrug resistance. The results are shown in FIGS. 28A-D (the number of viable cells $\times 10^4$ /ml is plotted against the concentration of catalase).

In FIG. 28, the following symbols represent 0, 5, 10, 15, 20 and 40 minutes of irradiation, respectively: $\circ$, $\diamond$, Δ , ∇ , $\triangleright$, and $\triangleleft$. FIGS. 28A and 28B show the results obtained using P388 and P388/ADR cells in PBS, respectively. FIGS. 28C and 28D show the results obtained using P388 and P388/ADR cells in RPMI 1640, respectively.

The results shown in FIG. 28 demonstrate that UVA irradiation has a greater growth inhibitory effect upon cells which are irradiated in RPMI 1640, which contains flavins

and amino acids, than upon cells which are irradiated in PBS. The addition of catalase to cell suspensions containing either PBS or RPMI 1640, protected the cells from damage (as judged by inhibition of growth) caused by protracted UVA irradiation. These results demonstrate that the addition of catalase to formulations of DOX containing flavins and/or amino acid photoenhancers protects UVA irradiated cells from damage due to the production of oxygen radicals.

In FIG. 29, it is shown that in the absence of UVA irradiation, catalase does not interfere with the growth inhibitory effect of DOX upon either the P388 or P388/ADR cell lines. For the results shown in FIG. 29, solutions containing increasing amounts of DOX in 0.2 ml PBS with or without 30 U/ml catalase were irradiated and then 1×10^5 viable cells in 0.8 ml complete growth medium were added to each well as described in Ex. 1. After 4 days in culture, the cell density was measured. In FIG. 29, the number of viable cells ($\times 10^4$ /ml) is plotted against the concentration of DOX present in the PBS solution during irradiation. The following symbols represent P388 cells grown in the presence of irradiated DOX solutions lacking catalase, P388/ADR cells grown in the presence of irradiated DOX solutions lacking catalase, P388 cells grown in the presence of irradiated DOX solutions containing 30 U/ml catalase and P388/ADR cells grown in the presence of irradiated DOX solutions containing 30 U/ml catalase, respectively: Δ , $\bullet$, $\circ$, and $\triangle$.

The above results demonstrate that catalase has no effect upon the growth inhibitory effect of DOX; however, these results show that catalase can protect cells from damage caused by protracted irradiation with UVA.

EXAMPLE 11

Formulation Of Solutions Containing Anthracyclines And Flavins

The preceding examples demonstrate that DOX in solution can be chemically changed (loss of growth inhibitory activity and absorbance spectrum) by long ultraviolet light in intensities that are used clinically (e.g., in the treatment of patients with psoriasis). To obtain this effect DOX (and other anthracycline drugs and/or other antineoplastic agents such as mitramycin, mitomycin C, taxol or Vinca alkaloids) are formulated with flavins (such as riboflavin, FMN, FAD) at molar quantity ratios of 1:80 to 1:1 flavin:DOX and the following enhancers:

- 1:50 to 1:500 DOX:DABCO molar ratio (or other tertiary aliphatic amines such as triethylamine); or
- 1:50 to 1:500 DOX:HEPES molar ratio (or other piperazines such as 1,4-dimethylpiperazine); or
- 1:1 to 1:6 DOX:tyrosine or tryptophan molar ratio (histidine, methionine or EDTA may be employed in place of tyrosine or tryptophan) and 1:50 to 1:500 DOX:piperazine (e.g., HEPES or 1,4-Dimethylpiperazine).

It has been reported that hydrogen peroxide and oxygen radicals may be formed when amino acids are exposed to UVA in the presence of photosensitizers. Accordingly, catalase (about 100 U/ml) and/or superoxide dismutase (50–300 U/ml) may be added to the above formulations. Superoxide dismutase (shown herein to act as a photoenhancer) converts superoxide radicals into hydrogen peroxide. Catalase converts hydrogen peroxide into H_2O and molecular oxygen. Recombinant human CuZn superoxide dismutase is available from Bio-Technology General Corp. (Iselin, N.J.).

These formulations of DOX, while not interfering with the systemic cytotoxic activity of the drug, allows the

destruction of DOX locally by clinically permissible quantities of long ultraviolet light (e.g., 3 to 5 mW/cm² for up to 20 minutes) will be delivered to the injection area once it is realized that extravasation has occurred (determined by swelling, pain and tissue staining by the drug).

EXAMPLE 12

Established murine and pig models are employed to demonstrate the prevention and/or treatment of DOX induced extravasation ulcers using the formulations and methods of the present invention.

a) Murine Model

An established murine model which allows the quantitation of DOX skin toxicity is used to demonstrate the effectiveness of the formulations and methods of [Dorr et al. (1980) *J. Pharmacol. Methods* 4:321 and Dorr et al. (1980) *Cancer Chemother. Pharmacol.* 5:17].

Dorsal hair is removed (3×3 cm area) from adult BALB/c mice (~25 g) (Jackson Laboratories, Bar Harbor, Me.) 24 hours prior to the ID injection of DOX solutions using a topical depilatory agent (e.g., Nect lotion). Animals are divided into groups of four each and are housed together for the duration of the experiment. Positive control injections contain 0.5 and 0.05 mg DOX diluted into 0.05 ml sterile normal (0.9%) saline (without preservatives); this dose corresponds to approximately 70 and 7 mg/m², respectively. Negative control injections comprise 0.05 ml sterile 0.9% saline (without preservatives). Test or experimental injections contain the DOX formulations described in Example 11.

Following ID injection, the injection area is exposed to UVA light using a suitable lamp assembly (e.g., Vilber-Lourmat lamps containing blacklight blue tubes T-40LN (Cole-Parmer Instrument Co., Niles, Ill.)) to permit the delivery of 3 to 5 mW/cm² for 0 to 20 minutes. The injected area is exposed to UVA light at 0 (i.e., immediately after injection), 1, 3 and 6 hours after injection. Following injection and exposure to UVA, all skin lesions are evaluated daily by measurement of the widest perpendicular width of ulceration, erythema and induration of the skin lesions are measured daily using a micrometer. The ulceration area×day (cm²×days) is calculated to quantitate an area under the toxicity-time curve (AUC) or the sum of total ulceration over time. In this mouse model, it has been shown that the above toxicity parameters increase in a DOX dose-dependent manner with respect to both the total area of the lesion (cm²/day) and the peak toxicity level (cm²) and the duration of toxicity (days). Statistical analyses (intergroup) may be conducted using standard techniques (e.g., Student's t-test or Student-Newman-Keuls multiple range test).

Desirable DOX formulations and UVA exposure levels are those which reduce or eliminate the DOX-induced lesions as compared to the positive control [i.e., they result in a statistically lower level of toxicity as compared to the control DOX solution (i.e., lacking either a PTC or a photoenhancer)].

b) Pig Model

An established swine model which allows the quantitation of DOX skin toxicity is used to demonstrate the effectiveness of the formulations and methods of the present invention for the treatment of injury caused by the extravasation of DOX [Averbuch et al. (1986) *J. Clin. Oncol.* 4:88]. In this swine model, it has been shown that DOX produces a predictable and reproducible skin ulceration and the total toxicity (AUC) increases in a DOX dose-dependent manner [Averbuch et al., supra].

Inbred weanling miniature white swine (~12 kg) (Veterinary Resources Branch, National Institutes of Health,

Bethesda, Md.) are anesthetized using standard techniques (e.g., an initial IM injection of ketamine, xylazine and atropine followed by intubation and administration of nitrous oxide, halothane and oxygen gas or administration of pentobarbital). DOX solutions are administered ID using a 25-gauge needle inserted approximately 4-5 mm into the skin. Each pig may receive multiple injections (up to 6 sites per midlateral aspect of each side) spaced 4-5 cm apart.

Positive control injections contain 1.0 to 2.0 mg DOX: a stock solution containing 2.0 mg/ml DOX in sterile 0.9% saline (may also contain lactose) is used for the injections. Negative control injections comprise 0.5 to 1.0 ml sterile 0.9% saline. Test or experimental injections contain the DOX formulations described in Example 11.

Following ID injection, the injection area is exposed to various doses of UVA light for various lengths of time as described above for the mouse model. Following injection and exposure to UVA, all skin lesions are measured using calipers 3 times per week for 2 weeks, followed by weekly measurements. Each animal is observed for an 8 week period. Areas of induration and skin ulceration are estimated by the product of the cross-perpendicular diameters. The total toxicity of the DOX-induced ulceration is calculated as the AUC in mm²×days. Statistical analyses (intergroup) may be conducted using standard techniques (e.g., Student's t-test or Student-Newman-Keuls multiple range test).

Desirable DOX formulations and UVA exposure levels are those which reduce or eliminate the DOX-induced lesions as compared to the positive control [i.e., they result in a statistically lower level of toxicity as compared to the control DOX solution (i.e., lacking either a PIC or a photoenhancer)].

From the above, it is evident that the anthracycline formulations of the present invention comprising a photosensitizer (e.g., a flavin), a photoenhancer (e.g., DABCO, piperazines, tyrosine, tryptophan, superoxide dismutase) and optionally catalase provide an improvement to present anthracycline formulations. These improved anthracycline formulations allow the prevention and/or treatment of injury due to extravasation of anthracyclines. In particular, these formulations when employed with the methods described herein (i.e., exposure to UVA) allow for the destruction of extravasated anthracyclines thereby preventing injury to the tissue surrounding the injection site.

I claim:

1. A method of treatment of extravasation injury, comprising:

a) providing:

i) a subject; and

ii) a formulation comprising a photoinactivation inducing compound; and

b) administering intravenously said formulation to said subject; and

c) exposing the intravenous administration site to UVA light.

2. The method of claim 1, wherein said formulation further comprises a vesicant chemotherapeutic.

3. The method of claim 2, wherein said chemotherapeutic is an antineoplastic drug.

4. The method of claim 3 wherein said antineoplastic is a Vinca alkaloid.

5. The method of claim 3, wherein said antineoplastic drug is an anthracycline.

6. The method of claim 5, wherein said anthracycline is doxorubicin.

7. The method of claim 1, wherein said photoinactivation inducing compound is a flavin.

8. The method of claim 7, wherein said flavin is selected from the group consisting of riboflavin, flavin mononucleotide, and flavin adenine dinucleotide.

9. The method of claim 1, wherein said formulation further comprises at least one photoenhancer compound.

10. The method of claim 9, wherein said photoenhancer compound is a tertiary aliphatic amine.

11. The method of claim 10, wherein said tertiary aliphatic amine is 1,4-diazabicyclo(2.2.2)octane.

12. The method of claim 9, wherein said photoenhancer compound is a piperazine.

13. The method of claim 12, wherein said piperazine is selected from the group consisting of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid and 1,4-dimethylpiperazine.

14. The method of claim 9, wherein said photoenhancer compound is selected from the group consisting of tyrosine, tryptophan, histidine, methionine, superoxide dismutase and ethylenediaminetetraacetic acid.

15. The method of claim 1, wherein said formulation further comprises catalase.

16. A composition comprising a serum-free saline solution comprising a vesicant chemotherapeutic, a photoinactivation inducing compound, and catalase.

17. A composition comprising vesicant chemotherapeutic, a photoinactivation inducing compound and at least one photoenhancer compound.

18. The composition of claim 17, wherein said vesicant chemotherapeutic is an antineoplastic drug.

19. The composition of claim 18, wherein said antineoplastic drug is an anthracycline.

20. The composition of claim 19, wherein said anthracycline is doxorubicin.

21. The composition of claim 17, wherein said photoinactivation inducing compound is a flavin.

22. The composition of claim 21, wherein said flavin is selected from the group consisting of riboflavin, flavin mononucleotide, and flavin adenine dinucleotide.

23. The composition of claim 17, wherein said photoenhancer compound is a tertiary aliphatic amine.

24. The composition of claim 23, wherein said tertiary aliphatic amine is 1,4-diazabicyclo(2.2.2)octane.

25. The composition of claim 17, wherein said photoenhancer compound is a piperazine.

26. The composition of claim 25, wherein said piperazine is selected from the group consisting of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid and 1,4-dimethylpiperazine.

27. The composition of claim 17, wherein said photoenhancer compound is selected from the group consisting of tyrosine, tryptophan, histidine, methionine, superoxide dismutase and ethylenediaminetetraacetic acid.

28. The composition of claim 17, further comprising catalase.

29. A method for preventing extravasation injury, comprising:

a) providing:

i) a subject; and

ii) a formulation comprising a photoinactivation inducing compound comprising a flavin and a vesicant chemotherapeutic; and

b) administering intravenously said formulation to said subject; and

c) exposing the intravenous administration site to UVA light.

30. The method of claim 29, wherein said chemotherapeutic is an antineoplastic drug.

5.780.446

33

31. The method of claim 30, wherein said antineoplastic drug is an anthracycline.

32. The method of claim 31, wherein said anthracycline is doxorubicin.

33. The method of claim 30, wherein said antineoplastic is a Vinca alkaloid.

34. The method of claim 29, wherein said flavin is selected from the group consisting of riboflavin, flavin mononucleotide, and flavin adenine dinucleotide.

35. The method of claim 34, wherein said formulation further comprises at least one photoenhancer compound.

36. The method of claim 35, wherein said photoenhancer compound is selected from the group consisting of tyrosine, tryptophan, histidine, methionine, superoxide dismutase and ethylenediaminetetraacetic acid.

34

37. The method of claim 35, wherein said photoenhancer compound is a tertiary aliphatic amine.

38. The method of claim 37, wherein said tertiary aliphatic amine is 1,4-diazabicyclo(2.2.2)octane.

39. The method of claim 37, wherein said photoenhancer compound is a piperazine.

40. The method of claim 39, wherein said piperazine is selected from the group consisting of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid and 1,4-dimethylpiperazine.

41. The method of claim 29, wherein said formulation further comprises catalase.

* * * * *

(12) SOLICITUD INTERNACIONAL PUBLICADA SEGÚN EL TRATADO DE COOPERACION DE PATENTES (PCT)

(19) Organización Mundial de Propiedad Intelectual
Oficina Internacional



(43) Fecha de Publicación

Marzo 21 de 2002 (21.03.2002)

PCT

(10) Número de Publicación Internacional

WO 02/22134 A1

(51) Clasificación Internacional de Patentes¹: A61K 31/66, 31/565, 31/704, A61P 35/00, A61K 9/08, 47/18

(21) Número de Solicitud Internacional: PCT/EP01/10398

(22) Fecha de Registro Internacional: Septiembre 07 de 2001 (07.09.2001)

(25) Idioma de registro: Inglés

(26) Idioma de Publicación: Inglés

(30) Información de Prioridad:

MI2000A001984 Septiembre 12 de 2000 (12.09.2000) IT

(81) Estados Designados (nacionales): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CQ, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Estados Designados (regionales): Patente ARIPO (GH, GM, KE, LS, MW, MZ, SD, SI, SZ, TZ, UG, ZW), patente Eurasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM) Patente Europea (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), Patente OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Publicado:

- con reporte de búsqueda internacional
- antes de la expiración del límite de tiempo para modificar las reivindicaciones y ser publicada de nuevo en el caso de recibo de modificaciones

(71) Solicitante: (Para todos los Estados designados excepto (Estados Unidos de América): PHARMACIA & UPJOHN SPA [IT/IT]; Via Robert Koch, 1.2, I-20152 Milan (IT).

Para los códigos de dos letras y otras abreviaturas, véase a "Las Notas Guías sobre Códigos y Abreviaturas" que aparecen al comienzo de cada emisión regular de la Gaceta PCT.

(72) Inventores; o

(75) Inventores/Solicitantes (para Estados Unidos de América solamente): MUGGETTI, Lorena [IT/IT]; Via Trento, 38, I-20036 Meda (IT). MARTINI, Alessandro [IT/IT]; Via Desiderio da Settignano, 14, I-20149 Milan (IT). BUZZI, Giovanni [IT/IT]; Via F. Poggi, 14, I-20131 Milan (IT). COLOMBO, Paolo [IT/IT]; Via Ludovico Cavaleri, 3, I-20147 Milan (IT).

(54) Título: "USO DE ARGININA EN LA PREPARACION DE UN MEDICAMENTO PARA LA PREVENCIÓN Y EL TRATAMIENTO DE LOS EFECTOS COLATERALES ASOCIADOS CON LA ADMINISTRACIÓN ENDOVENOSA DE PRODUCTOS FARMACEUTICOS"

(57) Resumen: La presente invención se refiere al uso de arginina y, más en particular, a las formulaciones inyectables para uso intravenoso que la contienen, en la prevención y tratamiento de efectos colaterales con la extravasación de fármacos administrados por vía intravenosa.

Pharmacia Italia
6-68823
98
95

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
21 March 2002 (21.03.2002)

PCT

(10) International Publication Number
WO 02/22134 A1 ✓

(51) International Patent Classification: A61K 31/66, 31/565, 31/704, A61P 35/00, A61K 9/08, 47/18

(21) International Application Number: PCT/EP01/10398 ✓

(22) International Filing Date:
7 September 2001 (07.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI2000A001984
12 September 2000 (12.09.2000) IT

(71) Applicant (for all designated States except US): PHARMACIA & UPJOHN SPA [IT/IT]; Via Robert Koch, 1.2, I-20152 Milan (IT).

(72) Inventors; and

(75) Inventors/Applicants (for US only): MUGGETTI, Lorena [IT/IT]; Via Trento, 38, I-20036 Meda (IT). MARTINI, Alessandro [IT/IT]; Via Desiderio da Settignano, 14, I-20149 Milan (IT). BUZZI, Giovanni [IT/IT]; Via F. Poggi, 14, I-20131 Milan (IT). COLOMBO, Paolo [IT/IT]; Via Ludovico Cavaleri, 3, I-20147 Milan (IT).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: USE OF ARGININE IN THE PREPARATION OF A MEDICAMENT FOR THE PREVENTION AND TREATMENT OF THE SIDE EFFECTS ASSOCIATED WITH THE INTRAVENOUS ADMINISTRATION OF PHARMACEUTICALS

(57) Abstract: The present invention relates to the use of arginine and, more in particular, to the injectable formulations for intravenous use comprising it, in the prevention and treatment of the side effects associated with the extravasation of drugs administered by intravenous route.

WO 02/22134 A1

99
96

TRATADO DE COOPERACION DE PATENTES
PCT
REPORTE DE EXAMEN PRELIMINAR INTERNACIONAL

(PCT Artículo 36 y Regla 70)

Registro de referencia del solicitante o agente 00340	PARA FUTURAS ACCIONES Ver Notificación o Transmisión del Reporte de Examen Preliminar Internacional (Formato PCT/IPEA/416)	
Solicitud Internacional No. PCT/EP 01/10398	Fecha de registro internacional (día/mes/año) 07/09/2001	Fecha de prioridad (día/mes/año) 12/09/2000
Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC A61K31/66		
Solicitante PHARMACIA ITALIA SPA et al.		

1. Este informe de examen preliminar internacional ha sido preparado por esta Autoridad Examinadora Preliminar Internacional y se transmite al solicitante de conformidad con el Artículo 36. 2. Este REPORTE consta de un total de <u>2</u> páginas, incluyendo la carátula. ☐ Este reporte también está acompañado de ANEXOS, es decir, páginas de descripción, reivindicaciones y/o dibujos que han sido modificados y son la base de este informe y/o páginas que contienen rectificaciones hechas ante esta Autoridad (ver Regla 70.16 y la Sección 607 de las Instrucciones Administrativas de conformidad con el PCT). Estos anexos constan de un total de _____ páginas.	
3. Este informe contiene indicaciones relacionadas con los siguientes ítems: I ☒ Bases del Reporte II ☐ Prioridad III ☒ No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad Industrial. IV ☐ Falta de unidad de invención V ☒ Afirmación razonada de conformidad con el Artículo 35(2) con respecto a novedad, nivel inventivo o aplicabilidad industrial; citaciones y explicaciones que sustentan dicha afirmación. VI ☐ Ciertos documentos citados VII ☐ Ciertos defectos en la solicitud internacional VIII ☐ Ciertas observaciones sobre la solicitud internacional	
Fecha de presentación de la demanda 22/03/2002	Fecha de terminación de este reporte 19/04/2002
Nombre y dirección de la autoridad internacional de examen preliminar: European Patent Office D-80298 Munich Tel. (+49-89) 2399 - 0, Tx: 523656 epmu d Fax: (+49- 89) 2399- 4465	Funcionario autorizado SEEGERT K Teléfono No. (+49-89) 2399 2828

Forma PCT/IPEA/409 (carátula) (Julio 1998)

I. Bases del Reporte

La base de este examen preliminar internacional es la solicitud como fue presentada originalmente.

III. No establecimiento de opinión respecto a la novedad, nivel inventivo y aplicabilidad industrial.

La cuestión de si la invención reivindicada parece ser novedosa, involucrar un nivel inventivo o es industrialmente aplicable no ha sido el objeto del examen preliminar internacional respecto a las reivindicaciones que no han sido buscadas (Artículo 17(2)(a) o (3) y Regla 66.1(e) del PCT); véase también el reporte de búsqueda internacional).

V. Declaración razonada según la Regla 66.2(a)(II) con respecto a la novedad, nivel inventivo o la aplicación industrial.

En el grado en que el examen preliminar internacional ha sido realizado (ver ítem III, arriba), lo siguiente es puntualizado:

A la luz de los documentos citados en el reporte de búsqueda internacional, se considera que la invención tal como es definida en las reivindicaciones, las cuales han sido objeto de un reporte de búsqueda internacional, cumple los criterios mencionados en el Artículo 33(1) del PCT, esto es, parece ser novedosa, involucrar un nivel inventivo y ser industrialmente aplicable.

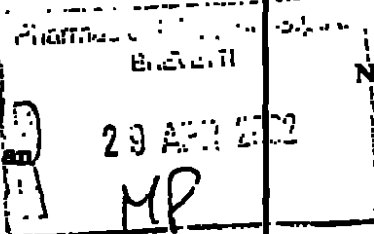
PATENT COOPERATION TREATY

401
98From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

PHARMACIA ITALIA SPA
Patent Department
Viale Pasteur, 10
I- 20014 Nerviano, Milan
ITALIE

NOTIFICATION OF TRANSMITTAL OF
INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing
(day/month/year)

24/04/2002

Applicant's or agent's file reference
00340

IMPORTANT NOTIFICATION

International application No.

PCT/EP 01/10398

International filing date (day/month/year)

07/09/2001

Priority date (day/month/year)

12/09/2000

Applicant

PHARMACIA ITALIA SPA et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.
4. **REMINDER**
The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/



European Patent Office
D-80298 Munich
Tel. (+49-89) 2399-0, Tx: 523656 eprmu d
Fax: (+49-89) 2399-4465

Authorized officer

LODOLO F
Tel. (+49-89) 2399 2828



PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 00340	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP 01/ 10398	International filing date (<i>day/month/year</i>) 07/09/2001	Priority date (<i>day/month/year</i>) 12/09/2000
International Patent Classification (IPC) or national classification and IPC A61K31/66		
Applicant PHARMACIA ITALIA SPA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 2 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 22/03/2002	Date of completion of this report 19/04/2002
Name and mailing address of the IPEA/  European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer SEEGERT K Tel. (+49-89) 2399 2828

Form PCT/IPEA/409 (cover sheet) (July 1998)



4

103
100

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No PCT/EP 01/ 10398

I. Basis of the report

The basis of this international preliminary examination is the application as originally filed.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question of whether the claimed invention appears to be novel, to involve an inventive step, or to be industrially applicable has not been the subject of the international preliminary examination in respect of the claims which have not been searched (Article 17(2)(a) or (3) and Rule 66.1(e) PCT); see also international search report).

V. Reasoned statement under Rule 66.2(a)(II) with regard to novelty, inventive step or industrial applicability

To the extent that the international preliminary examination has been carried out (see item III above), the following is pointed out:

In light of the documents cited in the international search report, it is considered that the invention as defined in the claims, which have been the subject of an international search report, meets the criteria mentioned in Article 33(1) PCT, i.e. appears to be novel, to involve an inventive step and to be industrially applicable.

PCT

De la OFICINA INTERNACIONAL

**NOTIFICACION DEL REGISTRO
DE UN CAMBIO**

(Regla 92 bis. 1 del PCT e
Instrucciones Administrativas, Sección 422)

Para:

PHARMACIA ITALIA S.P.A.
Patent Department
Viale Pasteur, 10
I-20014 Nerviano
ITALIA

Fecha de envío por correo (día/mes/año)
09 de Abril de 2002 (09.04.02)

Archivo de referencia del solicitante o agente
340

NOTIFICACION IMPORTANTE

Solicitud Internacional No.
PCT/EP01/10398

Fecha de registro internacional (día/mes/año)
07 de Septiembre de 2001 (07.09.01)

1. Las siguientes indicaciones aparecieron en el registro en relación con:

☒ el solicitante ☐ el inventor ☐ el agente ☐ el representante común

Nombre y Dirección
PHARMACIA & UPJOHN S.P.A.
Via Robert Koch, 1.2
I-20152 Milan
Italia

País de Nacionalidad
IT

País de Residencia
IT

No. Teléfono
39 02 4838 5416

No. Facsímile
3902 4838 5397

No. Teleimpresora

2. La Oficina Internacional por el presente notifica al solicitante que el siguiente cambio se ha registrado en relación con:

☐ la persona ☒ el nombre ☐ la dirección ☐ la nacionalidad ☐ la residencia

Nombre y Dirección
PHARMACIA ITALIA S.P.A.
Via Robert Koch, 1.2
I-20152 Milan
Italia

País de Nacionalidad
IT

País de Residencia
IT

No. Teléfono
+39 02 4838 5416

No. Facsímile
+3902 4838 5397

No. Teleimpresora

3. Otras observaciones, si fuese necesario:

Por favor note también el cambio en la dirección de la correspondencia como se indicó en la parte superior de esta forma.

4. Se ha enviado una copia de esta notificación a:

☒ la Oficina que recibe ☒ las Oficinas designadas relacionadas
☐ la Autoridad de Búsqueda Internacional ☐ las Oficinas seleccionadas relacionadas
☐ la Autoridad de Examen Preliminar Internacional ☐ otros:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsímile No.: (41-22) 740.14.35

Funcionario autorizado
Erich LORIS

Teléfono No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

PHARMACIA ITALIA S.P.A.
Patent Department
Viale Pasteur, 10
I-20014 Nerviano
ITALIE

EPO - DG 1

19. 04. 2002

(37)

Date of mailing (day/month/year)
09 April 2002 (09.04.02)Applicant's or agent's file reference
340

IMPORTANT NOTIFICATION

International application No.
PCT/EP01/10398International filing date (day/month/year)
07 September 2001 (07.09.01)

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address

PHARMACIA & UPJOHN S.P.A.
Via Robert Koch, 1.2
I-20152 Milan
Italy

State of Nationality

IT

State of Residence

IT

Telephone No.

39 02 4838 5416

Facsimile No.

3902 4838 5397

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☒ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address

PHARMACIA ITALIA S.P.A.
Via Robert Koch, 1.2
I-20152 Milan
Italy

State of Nationality

IT

State of Residence

IT

Telephone No.

39 02 4838 5416

Facsimile No.

3902 4838 5397

Teleprinter No.

3. Further observations, if necessary:

Please also note the change of the address of correspondence as indicated on the top of this form.

4. A copy of this notification has been sent to:

☒ the receiving Office ☒ the designated Offices concerned
☐ the International Searching Authority ☐ the elected Offices concerned
☐ the International Preliminary Examining Authority ☐ other:The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Erich LORIS

Telephone No.: (41-22) 338.83.38

206
103

**USO DE ARGININA EN LA PREPARACION DE UN MEDICAMENTO PARA LA
PREVENCION Y EL TRATAMIENTO DE LOS EFECTOS COLATERALES
ASOCIADOS CON LA ADMINISTRACION ENDOVENOSA DE PRODUCTOS
FARMACEUTICOS**

RESUMEN - OBJETO Y FINALIDAD

La presente invención se refiere a la utilización de arginina y, más en particular, a las formulaciones inyectables para uso endovenoso que la comprenden, para la prevención y tratamiento de los efectos colaterales asociados con la extravasación de drogas administradas por vía endovenosa.

USO DE ARGININA EN LA PREPARACION DE UN MEDICAMENTO PARA LA
PREVENCION Y EL TRATAMIENTO DE LOS EFECTOS COLATERALES
• ASOCIADOS CON LA ADMINISTRACION ENDOVENOSA DE PRODUCTOS
FARMACEUTICOS

La presente invención se refiere al uso de arginina y, más en particular, a sus formulaciones inyectables, para la prevención y tratamiento de los efectos colaterales asociados con la administración endovenosa de algunos productos farmacéuticos.

Se sabe que en terapia varias drogas administradas por vía endovenosa pueden causar, al menos potencialmente, daños vasculares en el lugar de inyección y daños ulcerativos en los tejidos circundantes, al producirse la extravasación.

Durante una administración intravascular, la extravasación se observa por derrame de fluido, habitualmente sangre, de una solución inyectada por vía endovenosa o una mezcla de ambas, de un vaso sanguíneo o linfático en el tejido perivascular circundante.

El fenómeno de la extravasación puede aparecer en varias situaciones, por ejemplo accidentalmente a través de una mala posición de la aguja dentro de la vena durante la administración endovenosa de la droga, con la consiguiente fuga de la solución medicada hacia el tejido perivascular circundante.

Alternativamente, es posible que se produzca la extravasación también en una vena demasiado pequeña, por ejemplo en relación con la velocidad de administración y/o el volumen de la solución inyectada, o cuando ya estuviera dañada por una administración previa de la droga o por un

trauma.

La extravasación de algunos fármacos administrados por vía endovenosa puede desembocar en ulceraciones particularmente dolorosas y extendidas que requieren, en algunos casos, la remoción quirúrgica del tejido interesado.

El fenómeno de extravasación asociado con la administración endovenosa de drogas, particularmente evidente en el caso de agentes antineoplásicos citotóxicos usados en la quimioterapia, se ha difundido ampliamente en la literatura. Véase, por ejemplo, Proc. Annu. Meet. Am. Soc. Clin. Oncol. 13: A1627, 1994; Seminars in Oncology, 27(3):347-61, 2000 Jun; Drug Safety, 12(4):245-255, 1995 Abr.; Nuritinga: An electronic journal of nursing ISSN 1440-1541 <http://www.healthsci.utas.edu.au/nursing/nuritinga/ios.html>

Aparte de los mencionados agentes quimioterapéuticos, otras clases de drogas, sintéticas o naturales, administradas por vía endovenosa, pueden ejercer este tipo de acción nociva si, luego de la extravasación, se ponen en contacto con tejidos perivascuales.

Nos referimos, por ejemplo, a drogas que tienen actividad antibiótica, antimicótica y también sedante.

Más en general, los compuestos que pueden causar daños ulcerativos luego de la extravasación comprenden agentes antineoplásicos tales como, por ejemplo, los antagonistas de tubulina, agentes alquilantes, antibióticos, antimetabolitos,

inhibidores de la topoisomerasa, inhibidores de la angiogénesis y derivados de platino. Además, los mencionados efectos colaterales pueden producirse también con otras drogas tales como, por ejemplo, agentes antivirales o vasosupresores y benzodiazepinas.

A este respecto, los ejemplos de compuestos específicos que pueden producir daños ulcerativos por extravasación incluyen, por ejemplo, amsacrina, vincristina, vinblastina, vinorelbina, vindesina, gemcitabina, etopósido, dacarbazina, estreptozocina, daunorrubicina, idarrubicina, epirubicina, doxorrubicina, alquilciclina (4-demetoxi-3'-deamino-3'-aziridinil-4'-metilsulfonil-daunorrubicina; código interno PNU 159548), plicamicina, penicilina, vancomicina, cloranfenicol, bleomicina, mitomicina, actinomicina D, paclitaxel, docetaxel, Sugén SU-5416, Sugén SU-6668, amfotericina B, cisplatino, carboplatino, ifosfamida; fluorouracilo, mecloretamina, mustina, carmustina, estramustina, irinotecan, topotecan, epinefrina, norepinefrina, dopamina, dobutamina.

Desde un punto de vista clínico no existen terapias consolidadas, y de cierta eficacia, para la prevención y tratamiento de aquellas lesiones/ulceraciones que, en los casos más serios y en ausencia de tratamientos adecuados, pueden avanzar hasta la necrosis de los tejidos interesados.

Sin embargo, se conocen en la técnica algunos remedios

posibles para disminuir la toxicidad local de algunas de las drogas más comunes que muestran una acción nociva luego de la extravasación. Véase, por ejemplo, la administración tópica de dimetilsulfóxido en el caso de la extravasación de antraciclina o mitomicina [Seminars in Oncology, 27(3):347-61, 2000 Jun.], la inyección local de hialuronidasa en el caso de alcaloides [Proc. Annu. Meet. Am. Chem. Soc. Clin. Oncol., 13:A1627, 1994] o la inyección local de tiosulfato de sodio en el caso de la mecloretamina [Drug Safety, 12(4):245-255, 1995 Abr.].

Como ya se informara, en los casos más desfavorables en los que los mencionados remedios no parecen ejercer el efecto deseado, la cirugía plástica sigue siendo la única posibilidad de intervención.

Con el objeto de minimizar los efectos no deseados asociados con la administración endovenosa de agentes terapéuticos capaces, al menos potencialmente, de causar daños ulcerativos por extravasación, se conocen en el arte formulaciones específicas. Véanse, por ejemplo, las formulaciones de antraciclina liposomal con un perfil de tolerabilidad mejorado en comparación con las formulaciones convencionales [Journal of Controlled Release, 53(1-3):275-9, 1998 Abr. 30] y el uso de ciclodextrinas en la preparación de formulaciones para la vía parenteral (US 5.804.568 a nombre de Supergen Inc).

Se sabe, sin embargo, que la encapsulación en liposomas, como así también la inclusión/asociación con ciclodextrinas de varios principios activos puede dar como resultado, en general, una notable variación del perfil farmacocinético de la sustancia terapéutica propiamente dicha.

Hemos descubierto ahora, inesperadamente, que la arginina (The Merck Index, XII Ed. No.817) resulta ser particularmente efectiva en la prevención y tratamiento de los efectos colaterales debidos a los fenómenos de extravasación asociados con la administración endovenosa de algunas drogas.

El uso de aminoácidos básicos y, más particularmente, de arginina, en la fabricación de formulaciones endovenosas de estramustina también se ha presentado en la solicitud de patente internacional WO 01/19372 a nombre de esta solicitante.

Sin embargo, como allí se manifiesta, la arginina actúa contra posibles tromboflebitis que, según se sabe, ocurren en el sitio de la inyección con las administraciones endovenosas de estramustina.

Es por lo tanto un objeto de la presente invención el uso de arginina, y de sus sales farmacéuticamente aceptables, en la preparación de un medicamento para la prevención y tratamiento de los efectos colaterales asociados con la extravasación de drogas administradas por vía endovenosa.

De acuerdo con una forma de realización preferida de la invención, el término arginina se refiere al aminoácido esencial en su forma ópticamente activa de L-arginina, opcionalmente en la forma de sal farmacéuticamente aceptable para la administración parenteral.

Las sales farmacéuticamente aceptables comprenden las sales de adición ácidas con ácidos orgánicos o inorgánicos tales como, por ejemplo, ácido clorhídrico, glutámico y aspártico.

Preferentemente, la presente invención se refiere al uso de arginina o clorhidrato de arginina.

Debido al perfil de seguridad y tolerabilidad que caracteriza a la arginina y sus sales farmacéuticamente aceptables, su amplia disponibilidad y versatilidad en el uso, la presente invención resulta ser especialmente ventajosa, en la terapia, en la administración endovenosa de varias drogas.

De acuerdo con una forma de realización preferida de la invención, la arginina resulta ser particularmente ventajosa en la prevención y tratamiento de daños perivasculares asociados con la administración endovenosa de drogas con actividad antitumoral, como por ejemplo antagonistas de tubulina, agentes alquilantes, antibióticos, antivirales, antimetabolitos, inhibidores de la topoisomerasa, inhibidores de la angiogénesis y derivados de platino.

A este respecto, se prefiere particularmente el uso de arginina en la terapia antitumoral que comprende la administración endovenosa de antraciclinas y derivados tales como la doxorubicina, epirubicina, idarrubicina, daunorubicina, alquiciclina (4-demetoxi-3'-deamino-3'-aziridinil-4'-metilsulfonil-daunorubicina; código interno PNU 159548), taxanos tales como paclitaxel y docetaxel; fosfato de estramustina; Sugén SU-5416 y Sugén SU-6668; como agentes individuales o en asociación con otros agentes quimioterapéuticos convencionales.

La arginina, de acuerdo con el objeto de la presente invención, puede ser administrada simultánea o secuencialmente con la administración de la droga a ser inyectada por vía endovenosa.

En el primer caso, la arginina puede estar presente como constituyente de la formulación propiamente dicha.

Como ejemplo, de acuerdo con el tipo de droga a ser administrada, la arginina puede estar presente en combinación con el principio activo, en forma de sal de arginina, o como ingrediente adicional, junto con cualesquier otros excipientes farmacéuticos para uso parenteral.

Un ejemplo típico de formulación capaz de prevenir los fenómenos ulcerativos que siguen a la posible extravasación de fosfato de estramustina, cuando se administra por vía endovenosa, es simplemente una formulación que comprende

fosfato de estramustina como la sal de arginina, como se expresa en los ejemplos.

Alternativamente, como se indica previamente, la arginina puede estar presente en la solución que contiene el principio activo a ser inyectado en forma endovenosa, como ingrediente adicional.

En ese caso, por ejemplo en la terapia que comprende la administración endovenosa del fosfato de estramustina, el principio activo puede estar constituido por una sal farmacéuticamente aceptable, por ejemplo la sal con N-metilglucamina, conocida también como meglumina.

Además de lo indicado previamente y debido al perfil de seguridad que caracteriza a la arginina, la misma puede estar presente como una sal en la formulación a ser inyectada, en combinación con el principio activo, y también como ingrediente adicional.

Al preparar dicha formulación, el especialista comprenderá fácilmente que se requiere más de un equivalente de arginina por equivalente de principio activo.

De lo anterior se desprende que pueden aplicarse consideraciones análogas para formulaciones de uso endovenoso que comprenden un principio activo que no sea el fosfato de estramustina y en todo caso capaz de causar fenómenos ulcerativos luego de la extravasación.

Como se indicara previamente, la arginina puede ser

también administrada en forma separada del principio activo, por ejemplo trabajando como se describe en la literatura para soluciones que contienen tiosulfato o ácido hialurónico de uso local luego de observarse los fenómenos de extravasación.

En ese caso, una solución de arginina fisiológica inyectable, opcionalmente en asociación con otros excipientes farmacéuticamente aceptables, puede ser administrada a través de la inyección local en la proximidad del área dañada por la administración endovenosa previa de la droga.

Entre los estudios pre-clínicos necesarios para una droga que ha de administrarse en forma endovenosa, se encuentra la evaluación de las reacciones locales que pudieran existir en caso de producirse una administración parcial accidental de la droga fuera del vaso propiamente dicho.

Dicho estudio, por ejemplo llevado a cabo con el modelo experimental descrito más abajo, permite evaluar la capacidad de irritación/daño histológico de drogas administradas por vía endovenosa, una vez extravasadas. Generalmente, aun cuando el examen clínico e histológico del sitio de inyección en los estudios toxicológicos repetidos permite dar una indicación de la tolerabilidad local a la droga, se prefiere de todos modos llevar a cabo un estudio ad hoc.

A este respecto, cualquier resultado obtenido en el

11

modelo animal es útil para entender si una posible extravasación accidental, en el uso clínico, puede producir los mencionados inconvenientes en el sitio de administración. Con el objeto de imitar cualquier posible situación clínica, el modelo que se considera usualmente es la administración paravenosa (vena marginal) en la oreja del conejo.

Como ejemplo, una cantidad limitada del compuesto a ser testeado (0,3-0,5 ml) se inyecta en el sitio perivasal; el sitio de inoculación se examina cuidadosamente durante al menos una semana.

Para evaluar posibles alteraciones en el sitio de inyección en la forma más correcta y objetiva posible, se usa un sistema de "puntaje".

Se presta especial atención a la presencia de eritema, edema inflamatorio y posibles apariciones de escaras, ulceraciones o lesiones con costras. Generalmente, cada animal es el control de sí mismo y la oreja opuesta recibe el vehículo, o sea la misma solución que no contiene el principio activo.

La concentración más elevada del compuesto a ser testeado es la concentración máxima pretendida en la práctica clínica.

Usualmente, se sacrifican dos animales: uno en la fase aguda, luego de la administración de la droga (48-72 horas) y otro más tarde, al menos una semana después de la

administración de la droga.

De esa manera, se lleva a cabo el examen histológico de todos los sitios de inyección.

Como se manifestara previamente, la arginina puede estar presente en la formulación a ser inyectada a los fines de prevenir y tratar los daños por extravasación, sea en combinación y/o asociación con uno o más principios activos o, alternativamente, per se, más excipientes fisiológicos convencionales.

Dichas formulaciones se preparan de acuerdo con técnicas convencionales usadas en la preparación de formas farmacéuticas para administración endovenosa y pueden contener también otros excipientes farmacéuticamente aceptables para uso parenteral tales como por ejemplo agentes para carga (p.ej. lactosa o manitol), reguladores de pH, antioxidantes, conservantes, reguladores de la tonicidad y similares.

Los siguientes ejemplos tienen como objeto ilustrar mejor la presente invención sin limitarla en forma alguna.

Ejemplo 1

Preparación de la sal de fosfato de estramustina con arginina

Se pesaron 300 mg de fosfato de estramustina en un cubilete y se dispersaron en 5 ml de agua bajo agitación magnética. Se agregaron a continuación 101 mg de base de arginina, bajo agitación, a la dispersión acuosa del

principio activo y, luego de pocos minutos, se obtuvo una solución clara.

La solución así preparada se diluyó luego con agua hasta un volumen final de 10 ml de modo de alcanzar una concentración final de 30 mg/ml de fosfato de estramustina y 10,1 mg/ml de arginina (relación molar 1:1, respectivamente).

Una solución preparada como se describe previamente, esterilizada apropiadamente por filtración, se testeó en materia de su tolerabilidad local en el animal, luego de la extravasación.

Ejemplo 2

Preparación de la sal de fosfato de estramustina con arginina, en mezcla con arginina

Se pesaron 300 mg de fosfato de estramustina en un cubilete y se dispersaron en 5 ml de agua bajo agitación magnética. Se agregaron a continuación 202 mg de base de arginina, bajo agitación, a la dispersión acuosa del principio activo y, luego de pocos minutos, se obtuvo una solución clara. El pH básico de la solución obtenida se llevó al valor fisiológico de alrededor de 7,5 mediante la adición lenta de ácido clorhídrico.

La solución así preparada se diluyó luego con agua hasta un volumen final de 10 ml de modo de alcanzar una concentración final de 30 mg/ml de fosfato de estramustina y 20,2 mg/ml de arginina (relación molar 1:2, respectivamente).

14

Una solución preparada como se describe previamente, esterilizada apropiadamente por filtración, se testeó en materia de su tolerabilidad local en el animal, luego de la extravasación.

Ejemplo 3**Preparación de la sal de fosfato de estramustina con N-metil-glucamina, en mezcla con arginina**

Se pesaron 300 mg de fosfato de estramustina en un cubilete y se dispersaron en 5 ml de agua bajo agitación magnética. Se agregaron a continuación 120,8 mg de N-metil-glucamina, bajo agitación magnética, a la dispersión del principio activo y, luego de un par de minutos, se obtuvo una solución clara. Se agregó entonces a la solución preparada, bajo agitación, una cantidad de arginina correspondiente a 202 mg usando una mezcla apropiada de base de arginina y clorhidrato de arginina de modo de mantener el pH final lo más cerca posible del pH fisiológico (alrededor de 7,5). La solución así preparada se diluye luego con agua hasta un volumen final de 10 ml de modo de alcanzar una concentración final de 30 mg/ml de fosfato de estramustina y 20,2 mg/ml de arginina (relación molar 1:2, respectivamente).

Se sometió a prueba una solución preparada como se describe previamente, debidamente esterilizada por filtración, en materia de su tolerabilidad en el animal luego de la extravasación.

Ejemplo 4

También se preparó la formulación descripta en el ejemplo anterior disolviendo la formulación liofilizada comercializada como Estracyt®, que contenía 300 mg del principio activo por frasco ampolla.

La reconstitución de la formulación se llevó a cabo usando 10 ml de una solución que contenía 20,2 mg/ml de arginina de modo de alcanzar una concentración final de 30 mg/ml de fosfato de estramustina y 20,2 mg/ml de arginina (relación molar 1:2, respectivamente).

La solución de arginina utilizada para disolver el liófilo comercial se preparó disolviendo en agua cantidades apropiadas de base de arginina y clorhidrato de modo de obtener una concentración final de 20,2 mg/ml y un pH lo más cercano posible al fisiológico (alrededor de 7,5).

Ejemplo 5

Preparación de una formulación con contenido de doxorubicina y arginina en una concentración molar de 1:1

Se pesaron 40 mg de clorhidrato de doxorubicina y 12 mg de arginina en un frasco de 20 ml. La mezcla se disolvió luego en 15 ml de solución fisiológica (NaCl 0,9% p/v) bajo agitación magnética. A la solución así obtenida se le agregó seguidamente una solución de HCl a pH=3. La solución así preparada se diluyó luego con la mencionada solución fisiológica hasta un volumen final de 20 ml de modo de

alcanzar una concentración final de 2 mg/ml de doxorubicina y 0,6 mg/ml de arginina (relación molar 1:1, respectivamente).

Una solución preparada como se describe previamente, debidamente esterilizada por filtración, se testeó en materia de su tolerabilidad local en el animal luego de la extravasación.

Ejemplo 6

Preparación de una formulación con contenido de doxorubicina y arginina en una concentración molar de 1:2

Trabajando en forma análoga a la descrita en el ejemplo 5 y usando una cantidad doble de arginina, o sea 24 mg de arginina por 40 mg de clorhidrato de doxorubicina, se preparó una solución que contenía doxorubicina y arginina en una relación molar de 1:2, respectivamente.

Una solución preparada como se describe previamente, debidamente esterilizada por filtración, se testeó en materia de su tolerabilidad local en el animal luego de la extravasación.

Ejemplo 7

Preparación de una formulación para uso endovenoso que contenía Sugan SU 5416 y arginina en una relación molar de 1:1

En un frasco graduado de 20 ml se diluyeron 10 ml de una solución acuosa de NaCl (0,9% p/v) con 10 ml de agua con el

objeto de obtener una solución al 0,45% p/v de cloruro de sodio.

39,79 mg de clorhidrato de arginina se disolvieron luego en la solución así obtenida mediante una simple agitación manual.

La solución solvente así preparada se utilizó luego para diluir una solución del compuesto Sugén SU 5416 que tenía la siguiente composición:

Componente	Cantidad en % (p/v) dentro de la formulación
Sugén SU 5416	0,45%
PEG 400	45%
Alcohol bencílico	2%
Cremophor EL	31,5%
Etolanol anhidro	q.b. a 1000

La dilución se llevó a cabo mezclando una parte de la formulación que contenía el principio activo con dos partes del solvente que contenía arginina de modo de obtener una solución que contenía Sugén SU 5416 y arginina en una relación molar de 1:1.

Una solución preparada como se describe previamente, debidamente esterilizada por filtración, se testeó en materia de su tolerabilidad local en el animal luego de la extravasación.

Ejemplo 8

Preparación de una formulación para vía endovenosa que contenía Sugén SU 5416 y arginina en una relación molar de 1:2

Trabajando en forma análoga a la expresada en el ejemplo 7, pero usando 79,58 mg de clorhidrato de arginina, se obtuvo una solución que contenía Sugén SU 5416 y arginina en una relación molar de 1:2, respectivamente.

Una solución preparada como se describe previamente, debidamente esterilizada por filtración, se testeó en materia de su tolerabilidad local en el animal luego de la extravasación.

REIVINDICACIONES

1. Utilización de arginina, y de sales farmacéuticamente aceptables de la misma, en la preparación de un medicamento para la prevención y tratamiento de los efectos colaterales asociados con la extravasación de drogas administradas por vía endovenosa.

2. Utilización de acuerdo con la reivindicación 1, caracterizada porque la arginina es en forma de base de arginina o su sal de clorhidrato.

3. Utilización de acuerdo con la reivindicación 1, caracterizada porque las drogas administradas por vía endovenosa son agentes antineoplásicos.

4. Utilización de acuerdo con la reivindicación 3, caracterizada porque los agentes antineoplásicos se seleccionan entre antagonistas de tubulina, agentes alquilantes, antibióticos, antivirales, antimetabolitos, inhibidores de la topoisomerasa, inhibidores de la angiogénesis y derivados de platino, usados individualmente o en combinación o en asociación con otros agentes quimioterapéuticos convencionales.

5. Utilización de acuerdo con la reivindicación 3, caracterizada porque el agente antineoplásico se selecciona entre doxorubicina, epirubicina, idarrubicina, daunorubicina, alquiciclina (4-demetoxi-3'-deamino-3'-aziridinil-4'-metilsulfonil-daunorubicina; código interno

PNU 159548), paclitaxel, docetaxel, fosfato de estramustina, Sugen SU-5416 y Sugen SU-6668, y sales farmacéuticamente aceptables de los mismos; utilizados como agentes individuales o en combinación o en asociación con otros agentes quimioterapéuticos convencionales.

6. Utilización de acuerdo con la reivindicación 5, caracterizada porque el agente antineoplásico es fosfato de estramustina, doxorubicina o Sugen SU 5416, y sales farmacéuticamente aceptables de los mismos.

7. Utilización de una composición para administración endovenosa, caracterizada porque comprende un agente antineoplásico y arginina, en la preparación de un medicamento antitumoral que previene y trata los efectos colaterales asociados con la extravasación de dicho agente antineoplásico.

8. Utilización de acuerdo con la reivindicación 7, caracterizada porque el agente antineoplásico se selecciona entre doxorubicina, Sugen SU 5416, fosfato de estramustina y sales farmacéuticamente aceptables de los mismos.

9. Utilización de acuerdo con la reivindicación 7, caracterizada porque la arginina es en forma de base de arginina o como una sal farmacéuticamente aceptable de la misma.

10. Utilización de acuerdo con la reivindicación 7, caracterizada porque el agente antineoplásico es en forma de

sal de arginina.

11. Un producto o kit para usar en una terapia antitumoral que comprende:

i) una formulación endovenosa de un agente antineoplásico;

y

ii) una formulación parenteral de arginina o una sal farmacéuticamente aceptable de la misma;

para la prevención o tratamiento de los efectos colaterales asociados con la extravasación de dicho agente antineoplásico.

12. Un método para prevenir y tratar los efectos colaterales asociados con la extravasación de un agente antineoplásico administrado por vía endovenosa que comprende la administración de dicho agente antineoplásico y arginina a un mamífero que lo necesite.

13. El método de acuerdo con la reivindicación 12, caracterizado porque la arginina está en forma de base de arginina o como sal farmacéuticamente aceptable de la misma.

14. El método de acuerdo con la reivindicación 12, caracterizado porque el agente antineoplásico se selecciona entre un antagonista de tubulina, agentes alquilantes, antibióticos, antivirales, antimetabolitos, inhibidores de la topoisomerasa, inhibidores de la angiogénesis y derivados de platino, usados individualmente o en combinación o en

asociación con otros agentes quimioterapéuticos convencionales.

15. El método de acuerdo con la reivindicación 14, caracterizado porque el agente antineoplásico se selecciona entre doxorubicina, epirubicina, idarrubicina, daunorubicina, alquiciclina (4-demetoxi-3'-deamino-3'-aziridinil-4'-metilsulfonil-daunorubicina; código interno PNU 159548), paclitaxel, docetaxel, fosfato de estramustina, Sugén SU-5416 y Sugén SU-6668, y sales farmacéuticamente aceptables de los mismos; utilizados como agentes individuales o en combinación o en asociación con otros agentes quimioterapéuticos convencionales.

16. El método de acuerdo con la reivindicación 15, caracterizado porque el agente antineoplásico es fosfato de estramustina, doxorubicina o Sugén SU 5416, y sales farmacéuticamente aceptables de los mismos.

17. El método de acuerdo con la reivindicación 12, caracterizado porque el agente antineoplásico está en forma de sal de arginina.

18. El método de acuerdo con la reivindicación 12, caracterizado porque el mamífero que lo necesita es un humano.

19. Una formulación para uso endovenoso que comprende arginina o una sal farmacéuticamente aceptable de la misma, y un agente antineoplásico seleccionado entre doxorubicina,

23

epirrubicina, idarrubicina, daunorrubicina, alquiciclina (4-demetoxi-3'-deamino-3'-aziridinil-4'-metilsulfonil-daunorrubicina; código interno PNU 159548), paclitaxel, docetaxel, fosfato de estramustina, Sugen SU-5416 y Sugen SU-6668, y sales farmacéuticamente aceptables de los mismos; utilizados como agentes individuales o en combinación o en asociación con otros agentes quimioterapéuticos convencionales.

REQUISITOS MINIMOS PARA ADMISION A TRAMITE

PATENTE DE INVENCION ☒

PCT

MODELO DE UTILIDAD ☐

- Indicación de que se solicita la concesión de una patente
- Datos de identificación del solicitante o de la persona que presenta la solicitud
- Descripción de la invención
- Dibujos de ser estos pertinentes
- Comprobante de Pago de las tasas establecidas

N/A
N/A
N/A
N/A
N/A

COMPLETA ☐

INCOMPLETA ☐

DISEÑO INDUSTRIAL ☐

- Indicación de que se solicita el registro de Diseño Industrial.
- Datos de identificación del solicitante o de la persona que presenta la solicitud.
- Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño.
- Comprobante de pago de las tasas establecidas.

[]
[]
[]
[]

COMPLETA ☐

INCOMPLETA ☐

ESQUEMA DE TRAZADO ☐

- Indicación de que se solicita el registro de un Esquema de trazado.
- Datos de identificación del solicitante o de la persona que presenta la solicitud.
- Representación gráfica del esquema de trazado
- Comprobante de pago de las tasas establecidas.

[]
[]
[]
[]

COMPLETA ☐

INCOMPLETA ☐

Firma Responsable

SUPERINDUSTRIA Y COMERCIO
Fecha Radicación: 2003-04-08 16:42:25
Folios: 128
Trámite: 011 PCT-PATENT 379 RFSE MAC? 411 PRESENTAC
Dependencia: 2000 DIVISION DE NUEVAS CREACIONES

Carrera 13 No. 27-00 Piso 5 y 10
Conmutador: 334 12 21-2-3-4
Fax: 281 31 25 e-mail: info@sic.gov.co
Santa Fe de Bogotá D.C. - Colombia