

Señora Jefe de la
DIVISION DE SIGNOS DISTINTIVOS
Superintendencia de Industria y Comercio
E. S. D.

SUPERINDUSTRIA Y COMERCIO
Radicación : 01087299 00000018 Folios: 76
Fecha (HNB): 2004-08-09 16:41:26
Trámite : 002 PATENTES y 1 REGISTRO/ 449 RESPUESTA
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Trámite : 002

Evento : 001

Actuación : 449

Referencia : Expediente No. 01.087.299
Asunto : Solicitud de patente de invención para:
**COMPOSICIONES INHIBIDORAS DE CICLOOXIGENASA-2
QUE TIENE RAPIDO ACCESO DE EFECTO TERAPEUTICO**
Solicitante : PHARMACIA CORPORATION
Opositor : TECNOQUÍMICAS S.A.

I. ALEGATOS CONTESTACIÓN OPOSICIÓN

Yo, Emilio FERRERO, en mi condición de apoderado de la sociedad solicitante de la patente de la referencia, por el presente escrito doy respuesta a la oposición formulada por el doctor José Luis REYES VILLAMIZAR, apoderado de la sociedad TECNOQUÍMICAS S.A., opositora en el asunto de la referencia.

II. CONTESTACIÓN DENTRO DEL TÉRMINO LEGAL

El Oficio No. 04851, en el cual su Despacho dispuso otorgar a mi representada término para presentar sus argumentaciones a favor de la patentabilidad del invento de que trata el asunto en referencia, me fue notificado por fijación en lista el día trece (13) de mayo de dos mil cuatro (2004). El término previsto en el artículo 43 de la Decisión 486 de la Comisión de la Comunidad Andina vence el doce (12) de agosto de

En efecto, en diversas oportunidades ha señalado el Tribunal, que el examinador deberá considerar las observaciones (hoy oposiciones) presentadas por quien tenga interés legítimo, entendido como tal, el tercero que pueda verse afectado por la concesión de una patente. Así lo determinó expresamente el Tribunal de Justicia de la Comunidad Andina en sentencia de interpretación prejudicial del 8 de mayo de 2002, Proceso 25-IP-2002, señalando:

"La figura de las observaciones constituye una eficaz ayuda para la autoridad competente, porque por medio de ella se ejerce el derecho de oposición de terceros que pueden verse afectados por la concesión de una patente que podría violentar derechos adquiridos o estar en contra de la normatividad vigente". (Resaltado fuera del texto).

Como podrá advertir su Despacho, en ninguna parte del escrito de oposición presentado ante su Despacho el 1º de julio de 2003 y en el escrito de sustentación presentado el 24 de septiembre de 2003, la sociedad **TECNOQUÍMICAS S.A.** opositora en el asunto de la referencia, presenta las razones que justifican su interés legítimo para ser consideradas por su Despacho como **afectada directamente en su interés por la solicitud de patente de mi representada.** En el presente caso, la opositora no ha dado cumplimiento al primer y básico requisito establecido en la norma citada para que su oposición sea considerada como válida en contra de la solicitud de mi representada.

Por tanto, solicito a su Despacho dar por no cumplido el requisito señalado y de plano declarar sin fundamento la oposición de **TECNOQUÍMICAS S.A.**

V. FUNDAMENTO FÁCTICO DE LA OPOSICIÓN

Con fundamento en la premisa señalada en el punto anterior, solicito a su Despacho no tomar en cuenta la oposición de la referencia para negar la solicitud de mi representada. Sin embargo, me referiré a los argumentos de fondo presentados por la opositora para concluir que no son

dos mil cuatro (2004). Por tanto, esta defensa está siendo presentada dentro de la oportunidad legal.

III. RESPUESTA A LAS PETICIONES

Me opongo a las peticiones de la sociedad opositora y en consecuencia, solicito a ese Despacho que con fundamento en las consideraciones de hecho y de derecho que se presentan en este escrito se declare sin fundamento la oposición presentada por **TECNOQUÍMICAS S.A.**

En consecuencia, se declare que el invento titulado **COMPOSICIONES INHIBIDORAS DE CICLOOXIGENASA-2 QUE TIENE RÁPIDO ACCESO DE EFECTO TERAPÉUTICO** cumple con los requisitos exigidos en el artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina y demás normas concordantes. Por tanto, solicito se sirva otorgar a mi representada la patente solicitada.

IV. AUSENCIA DE CUMPLIMIENTO DEL REQUISITO DEL LEGÍTIMO INTERES EN LA OPOSITORA

De conformidad con lo dispuesto en el artículo 42 de la Decisión 486 de la Comisión de la Comunidad Andina, quien tenga interés legítimo puede presentar oposición para desvirtuar la patentabilidad de la invención.

A este respecto llamo la atención de su Despacho a la interpretación que en diversas oportunidades ha hecho el Tribunal Andino de Justicia de la Comunidad Andina en cuanto al requisito del interés legítimo del opositor que consagraba el artículo 25 de la Decisión 344, y que en igual forma exige el artículo 42 de la Decisión 486 de la Comisión de la Comunidad Andina.

aplicables a la solicitud de mi representada y que por tanto, no constituyen fundamento válido para señalar que el invento de que trata la solicitud de la referencia no cumple con los requisitos establecidos en los artículos 14, 16 y 18 de la Decisión 486 de la Comisión de la Comunidad Andina. Lo anterior por cuanto el señalamiento que hace la opositora de la falta de novedad y de nivel inventivo para el invento para el cual pretende mi representada la concesión de la patente es errado, carece de sustento fáctico y por tanto de sustento de derecho para que prospere en contra de la solicitud de mi representada.

En efecto, en el escrito de oposición señala el apoderado de la opositora, que la solicitud de mi representada no cumple con los requisitos de los artículos 14, 16 y 18 de la Decisión 486, por cuanto:

- 5.1. Antes de la fecha de prioridad se conocían los inhibidores de ciclooxigenasa-2, sus propiedades y características farmacocinéticas, la posibilidad de su administración por vía oral, los rangos de ingrediente activo en las formulaciones, las dosis unitarias, los excipientes, los usos, y la incidencia directa del tamaño de partícula sobre los parámetros farmacocinéticos de un fármaco.
- 5.2 La eventual biodisponibilidad mejorada de una composición de inhibidores de la COX-2 por obra de la presencia de tal familia de principios activos en los tamaños de partícula revelados, corresponde simplemente a un "fenómeno que es conocido, que no se traduce en ningún avance sobre el estado de la técnica y que es el resultado de un proceso rutinario de pre-formulación, consistente en realizar ensayos sobre disolución empleando tamaños de partícula diversos".
- 5.3 El invento de la referencia carece de novedad y nivel inventivo por cuanto en las patentes US 5,466,823, US

5,474,995 y US 5,633,272 ya se hacía clara mención al uso de compuestos fenil heterocíclicos, como Celecoxib, Rofecoxib y Valdecoxib, respectivamente, en el tratamiento de la inflamación y desórdenes relacionados.

En consecuencia, la opositora solicita a su Despacho negar la concesión del privilegio de patente solicitada por mi representada.

VI. RESPUESTA A LOS HECHOS Y ALEGATOS

A continuación presento a su Despacho las razones que demuestran que contrario a lo señalado por el apoderado de la sociedad opositora, el invento para el cual se solicita la protección a través de la concesión de la patente en el expediente de la referencia, cumple cabalmente con los requisitos del artículo 14 y demás normas concordantes de la Decisión 486 de la Comisión de la Comunidad Andina.

Además, los argumentos que aquí se presentan demuestran que los fundamentos de la opositora carecen de fundamento válido y por tanto, no pueden ni deben ser tomados en cuenta para negar la patente solicitada.

En efecto, me referiré a cada una de las alegaciones que en contra de la patentabilidad del invento titulado **COMPOSICIONES INHIBIDORAS DE CICLOOXIGENASA-2 QUE TIENE RÁPIDO ACCESO DE EFECTO TERAPÉUTICO** presenta la opositora:

6.1. Ausencia de novedad y nivel inventivo:

La opositora alega que reducir el tamaño de partícula de un medicamento para lograr una mejor biodisponibilidad no constituye nivel inventivo. El argumento de la opositora presupone, sin embargo, que la reducción del tamaño de partícula siempre resulta en una biodisponibilidad más alta. Esto no es así. Por lo tanto, este argumento contra

la patentabilidad de la invención reivindicada se fundamenta en una premisa falsa y se le debe dar poco mérito, por las siguientes razones:

6.1.1 La biodisponibilidad es el resultado de muchos factores además de las tasas de disolución, incluyendo absorción, extracción hepática, fracción libre en plasma e incluso peso molecular. Al respecto solicito tener en cuenta los siguientes artículos:

- Martínez & Amidon, "A mechanistic approach to understanding the factors affecting drug absorption: A review of Fundamentals", J. Clin. Pharmacol, 42, 620-643, 2002 (Prueba 9.1.) en página 620, parágrafo que hace el puente entre las columnas 1 y 2;
- Pang, "Modeling of intestinal drug absorption: Roles of transporters and metabolic enzymes", Drug Metab. Disposit, 31, 1507-1519, 2003 (Prueba 9.2) en página 1507, renglón 1 a página 1509, columna 1, renglón 16;
- Kushida et al., "Improvement of dissolution and oral absorption of ER-34122, A poorly water-soluble dual 5-Lipoxygenase/Cyclooxygenase inhibitor with anti-inflammatory activity by preparing solid dispersion", J. Pharm. Sci. 91, 258-266, 2002 (Prueba 9.3) en página 261, columna 1, renglones 1-11 y Gráfica.

Así, no se puede presumir que la reducción del tamaño de partícula para lograr tasas de disolución más rápidas necesariamente incrementa la biodisponibilidad.

6.1.2 El efecto de la reducción del tamaño de partícula sobre la biodisponibilidad no se puede predecir con certeza para ningún medicamento particular. De hecho, hay numerosos ejemplos, previos a la fecha de presentación, de medicamentos que tienen una solubilidad acuosa baja para las cuales la reducción del tamaño de la partícula no resulta en una mayor biodisponibilidad. Cito los siguientes artículos:

- 1 • Richter & Terhaag, "The relative bioavailability and pharmacokinetics of carbamazepine", Int. J. Clin. Pharmacol. 16, 377-79, 1978, (Prueba 9.4).

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Richter & Terhaag encontraron que la biodisponibilidad de dos preparaciones de carbamazepina (carbamazepina 1 y carbamazepina 2) que tenían dos distribuciones diferentes de tamaños de partícula (véase la Figura 3) no era la que ellos esperaban:

"Encontramos una biodisponibilidad de carbamazepina 1 más baja en cada uno de los 3 sujetos. Este resultado no pudo haber sido causado por inducción del metabolismo de carbamazepina porque los experimentos se realizaron en un estudio cruzado y no se cambiaron los tiempos de vida media.

Pero la distribución de tamaños de partícula era diferente. La carbamazepina 1 sólo tiene una pequeña variación en los tamaños de partícula, mientras que la carbamazepina 2 está conformada por partículas de diámetros muy diferentes. Desde este punto de vista, uno podría esperar que la carbamazepina 1 se disolviera y absorbiera más bien rápido y más completamente que la carbamazepina 2. Pero en la práctica encontramos un tiempo de reabsorción más largo en la carbamazepina 2 y por lo tanto una mayor biodisponibilidad.

A partir de estos hechos se debe concluir que hay otras propiedades desconocidas de los cristales de carbamazepina que afectan la biodisponibilidad." (Página 378, col 2, renglón 14, a página 379, col. 2, renglón 5.)

- 2 • Shastri et al., "Relationship among particle size distribution profile, plasma values and anthelmintic efficacy of oxfendazole", Am. J. Vet. Res. 41, 2095-2101, diciembre de 1980, (Prueba 9.5). Se probó la biodisponibilidad de tres suspensiones por lo demás idénticas, cada una con un tamaño diferente de partícula de oxfendazol. De nuevo, la reducción del tamaño de partícula no siempre aumenta la biodisponibilidad:

"Los valores del área en plasma indicaron que la suspensión C (dw = 12.0 mm) estuvo significativamente ($P < 0.05$) menos biodisponible de lo que estuvo la suspensión A (dw = 1.65 mm); no hubo ninguna diferencia

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entre la suspensión A y la suspensión B [3.2 mm].” (Página 2095, col. 1, primer párrafo. El resultado es nuestro).

- 3 • Kohno et al., “Pharmacokinetics and bioavailability of Diltiazem (CRD-401) in Dog”, Drug Res. 27, 1424-28, 1977, (Prueba 9.6). Se midió la biodisponibilidad de tres preparaciones diferentes de clorhidrato de diltiazem que tenían tres tamaños de partícula diferentes (cristal I, 400-500 mm; cristal II, 10-100 mm; y cristal en polvo I, 5-30 mm), página 1425, col. 1, párrafo 2.1. y se concluyó:

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“En el estudio de biodisponibilidad, una comparación de concentraciones de plasma de diltiazem entre los dos cristales diferentes y el polvo micronizado no indicó ninguna diferencia en su biodisponibilidad, cuando se administraron en forma de cápsulas.

[...]

“Los valores de área bajo la curva de tiempo de concentración en plasma (ABC), nivel máximo de plasma (Cpmax) y el tiempo necesario para alcanzar su pico (tmax) se resumieron en la Tabla 3. En el cristal I, el nivel máximo medio en plasma fue 16.2 +/- 3.7 mg/dl, el cual se alcanzó 1 hora después de la administración. Este nivel pico fue comparable con el nivel obtenido después de la administración del cristal II, mientras que el nivel máximo medio en plasma para el cristal I en forma de polvo fue 17.6 +/- mg/dl y pareció ser un poco más alto que el de las otras dos formas de cristal. Sin embargo, no se detectaron diferencias significativas en los niveles pico en plasma entre las tres formas de dosificación probadas (p < 0.1). En cuanto a la biodisponibilidad, estas tres formas de dosificación de prueba produjeron casi la misma área bajo la curva de tiempo de concentración en plasma, por lo cual no se sugiere una biodisponibilidad diferente del diltiazem entre estas tres formas. Esta observación se confirmó estadísticamente después, mediante análisis de varianza (p > 0.1)”. (Resumen y párrafo que une las páginas 1427 y 1428. El resultado es nuestro).

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Publicaciones posteriores a la fecha de presentación demuestran la misma incertidumbre con respecto al tamaño de partícula y la biodisponibilidad. Véase por ejemplo en Andrýsek, “Impact of physical properties of formulations on bioavailability of active substance: current and novel drugs with cyclosporine”, Mol. Immunol. 39, 1061-65, 2003, (Prueba 9.7). Andrýsek probó la bioequivalencia de

varias formas de dosificación oral de ciclosporina. Andrysek dice:

"Este estudio clínico mostró que las formulaciones A y B cumplen los criterios de bioequivalencia en relación con la formulación C.

Por otra parte, la comparación del tamaño efectivo de partículas creadas después de la disgregación mostró que las formulaciones A y B dan partículas aproximadamente 1000 veces más grandes que la formulación C de referencia.

Estas conclusiones son contrarias al concepto anterior en cuanto a que más pequeño es mejor." (Página 1063, columna 2, renglones 3-10. El resultado es nuestro).

Más aún, como lo enseña la presente especificación, los efectos de la reducción del tamaño de partícula desde el rango de micropartícula hasta el rango de nanopartícula generalmente son impredecibles en un medicamento o clase particular de medicamentos. Ford et al., "Nanospheres of cyclosporin A: poor oral absorption in dogs", Intl. J. Pharmaceutics 183, 3-6, 1999, (Prueba 9.8), compararon la biodisponibilidad relativa de nanoesferas de ciclosporina A (CYA) pura (es decir, "que sólo comprenden el medicamento mismo", p.3) con la de una formulación de microemulsión. Ford et al. encontraron que la biodisponibilidad relativa de CYA en las nanoesferas "era sólo del 3% en comparación con el área bajo la curva de tiempo de concentración en plasma", (resumen). Ford et al. concluyen que la ciclosporina se puede formar fácilmente en nanopartículas, "[s]in embargo, la capacidad de este sistema para facilitar la absorción oral de CYA en perros fue pobre en comparación con una formulación de microemulsión de absorción eficiente", (página 6, último párrafo).

Ciertamente, incluso dentro del rango de nanopartícula puede haber resultados inesperados. Por ejemplo, la presente especificación enseña que las partículas de celecoxib en el rango de 500-900 nm son equivalentes o casi equivalentes en Tmax, Cmax y ABC a partículas más pequeñas del rango 200-400 nm. Véase la Tabla 2 de la especificación, en la página 47.

Andrýsek (Prueba 9.7) no encontró ninguna relación entre el tamaño de la partícula y la biodisponibilidad de la ciclosporina en el rango de las nanopartículas:

"Mientras tanto, se realizaron experimentos biofarmacéuticos [sic] enfocados a la correlación que hay entre la distribución del tamaño de las partículas y la biodisponibilidad en perros. Estos experimentos mostraron que la misma biodisponibilidad mejorada se puede lograr con sistemas que dan una distribución de tamaño de partícula casi en el rango del submicrón. En consecuencia, no se observó una correlación entre la distribución del tamaño de partícula con respecto al peso molecular de agregación y la biodisponibilidad cuando se evaluaron sistemas microdispersos modelo con tamaño de partícula de 141nm (309 y 1001nm)". (Párrafo entre las páginas 1063 y 1064, referencias internas omitidas. El resultado es nuestro).

Las enseñanzas de cada una de estas referencias son directamente contrarias a lo que alega el opositor en cuanto a lo que se esperaría si se redujera el tamaño de la partícula del medicamento. Es claro, tal como lo prueban las enseñanzas de estas referencias, que uno no puede presumir que la reducción del tamaño de partícula del medicamento resultará en un mayor biodisponibilidad.

Los hechos anteriormente mencionados, los cuales se encuentran debidamente probados, permiten concluir que los documentos citados como fundamento de la oposición, esto es las solicitudes US 5,466,823, US 5,474,995 y US 5.633,272, no afectan los requisitos de novedad y nivel inventivo de la solicitud de patente que se tramita en el expediente de la referencia.

- 6.1.3 De esta manera, se concluye que contrario a lo señalado por la sociedad opositora la presente invención también cumple con el requisito de patentabilidad en cuanto tiene nivel inventivo de acuerdo con lo establecido en el artículo 18 de la Decisión 486 de la Comisión de la Comunidad Andina.

Por lo anterior, y tal como quedó demostrado, el cargo realizado en cuanto a la ausencia de novedad y nivel inventivo de la invención solicitada en patente por mi representada, carece totalmente de fundamento y por tanto, solicito a ese Despacho así declararlo.

6.2. Ausencia de claridad de las reivindicaciones:

La opositora alega que las reivindicaciones 1-12 y 16-18 carecen de especificidad. Para ello mi representada ha eliminado las reivindicaciones 2, 3, 8-11 y 18 y se han modificado las reivindicaciones 1, 4, 6, 12, 13, 16 y 17.

La reivindicación 1 ha sido modificada para que comprenda la fórmula de la reivindicación 11 ahora eliminada. La reivindicación 1 modificada es específica porque describe una fórmula con sustituyentes particulares y definidos.

En cuanto a la ausencia de especificidad de las reivindicaciones, que alega el opositor, por considerar que no describen otros componentes de la composición reivindicada o sus porcentajes, señalo que las reivindicaciones se dirigen a una composición farmacéutica que comprende el agente terapéutico indicado (es decir, un inhibidor de ciclooxigenasa-2 selectivo que tiene la fórmula descrita) que tiene un tamaño de partícula del rango descrito. Puesto que la invención radica en el agente terapéutico descrito y su tamaño de partícula, el solicitante tiene derecho a reivindicar todas las composiciones farmacéuticas que contengan el ingrediente descrito. Si se obligara al solicitante a que especificara ingredientes adicionales y sus porcentajes se limitaría indebidamente la protección a la cual el solicitante tiene derecho.

Más aún, una persona versada reconocería inmediatamente que dichas composiciones pueden incluir uno o más excipientes farmacéuticamente aceptables y uno o más

agentes terapéuticos adicionales. Véase la página 13 de la traducción al español del resumen de la invención, parrafo 3. Así, una persona versada no encontraría que las reivindicaciones carecen de especificidad porque no especifican ingredientes (opcionales) adicionales.

En consecuencia, quedan desvirtuadas las afirmaciones de la opositora en contra de la patentabilidad del invento para el cual solicita la protección mi representada.

VII. MODIFICACIÓN DE LA SOLICITUD

Tal como lo indiqué en el capítulo anterior, junto con este escrito presento Formulario de Modificaciones y Correcciones, con nuevo capítulo reivindicatorio el cual solicito sea tenido en cuenta por ese Despacho.

VIII. PETICIONES

Con fundamento en lo anterior solicito a ese Despacho lo siguiente:

- 8.1. Declarar infundada la oposición presentada por **TECNOQUÍMICAS S.A.**
- 8.2. Declarar que el invento titulado **"COMPOSICIONES INHIBIDORAS DE CICLOOXIGENASA-2 QUE TIENE RÁPIDO ACCESO DE EFECTO TERAPÉUTICO"** para el cual mi representada solicita el otorgamiento de la patente de invención cumple con los requisitos del artículo 14 y concordantes de la Decisión 486 de la Comisión de la Comunidad Andina.
- 8.3. Conceder la patente para el invento **"COMPOSICIONES INHIBIDORAS DE CICLOOXIGENASA-2 QUE TIENE RÁPIDO ACCESO DE EFECTO TERAPÉUTICO"** a nombre de mi representada **PHARMACIA CORPORATION.**

IX. PRUEBAS

Atentamente solicito a ese Despacho se sirva tener como prueba a favor de mi representada las siguientes:

- 9.1. Martínez & Amidon, "A mechanistic approach to understanding the factors affecting drug absorption: A review of Fundamentals", J. Clin. Pharmacol. 42, 620-43, 2002.
- 9.2. Pang, "Modeling of intestinal drug absorption: Roles of transporters and metabolic enzymes", Drug Metab. Disposit. 31, 1507-19, 2003.
- 9.3. Kushida et al., "Improvement of dissolution and oral absorption of ER-34122, A poorly water-soluble dual 5-Lipoxygenase/Cyclooxygenase inhibitor with anti-inflammatory activity by preparing solid dispersion", J. Pharm. Sci. 91, 258-66, 2002.
- 9.4. Richter & Terhaag, "The relative bioavailability and pharmacokinetics of carbamazepine", Int. J. Clin. Pharmacol. 16, 377-79, 1978.
- 9.5. Shastri et al., "Relationship among particle size distribution profile, plasma values and anthelmintic efficacy of oxfendazole", Am. J. Vet. Res. 41, 2095-2101, diciembre de 1980,
- 9.6. Kohno et al., "Pharmacokinetics and bioavailability of Diltiazem (CRD-401) in Dog", Drug Res. 27, 1424-28, 1977
- 9.7. Andrýsek, "Impact of physical properties of formulations on bioavailability of active substance: current and novel drugs with cyclosporine", Mol. Immunol. 39, 1061-65, 2003

- 9.8 Ford et al., "Nanospheres of cyclosporin A: poor oral absorption in dogs", Intl. J. Pharmaceutics 183, 3-6, 1999,
- 9.9. Todos y cada uno de los documentos que obran en el expediente y que reposan en los archivos del Despacho a su cargo.

X. ANEXOS

Anexos a este escrito los siguientes documentos:

- 10.1. Los documentos enunciados en los numerales 9.1. a 9.8. del acápite de pruebas.
- 10.2. Formulario de Modificaciones y Correcciones con el cual se presenta nuevo capítulo reivindicatorio y el recibo de pago por concepto de la tasa correspondiente para modificaciones.

XI. NOTIFICACIONES

Mi representada y yo recibiremos notificaciones en la Secretaría de su Despacho o en mi oficina de abogado ubicada en la Carrera 4ª No. 72-35 de esta ciudad de Bogotá, D.C.

Señora Jefe,



EMILIO FERRERO

T.P.A. No. 31.929

COLO 12482-234-002-0

COLO 12482-801-004-9

JGG/JGG/ID-000099/WPC12482

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Folios: 76

SUPERINDUSTRIA Y COMERCIO

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 Dependencia: 2029 DIVISION DE NUEVAS CREACIONES

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 04 - 60,314
 FECHA : AGOSTO 2 DE 2004

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
CAVELIER ABOGADOS	CONSIGNACION	BANCO POPULAR	050-00110-6	23490084	02/08/2004	17,010,800.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-03	CAMBIO DE MODALIDAD Y MODIFICA	
12		MARCAS Y LEYAS COMERCIALES	87,000.00
		TOTAL :	87,000.00

SON: OCHENTA Y SIETE MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

Cdo 12482-234-002-0

**Industria y Comercio**
SUPERINTENDENCIA

Espacio reservado para el adhesivo de radicación

FORMULARIO UNICO DE CORRECCIONES Y MODIFICACIONES
2000-10

1

SOLICITUD DE:

☐ Corrección de Error Material

☒ Modificación a una solicitud

2

SOLICITANTE

Nombre: **PHARMACIA CORPORATION**
sociedad constituida y existente bajo las leyes del Estado de Delaware, Estados Unidos de América

Dirección: 5200 Old Orchard Road, Skokie, Illinois 60077, Estados Unidos de América

Teléfono: 546 09 70
E-mail: clientes@camyp.com
Fax: 249 40 70
Domicilio: Skokie, Illinois, Estados Unidos de América

IDENTIFICACION

C.C. ☐ NIT ☐

C.E. ☐ Otro ☐

Número: _____

3

REPRESENTANTE APODERADO

Nombre: **Emilio FERRERO**

Dirección: Carrera 4ª No. 72-35
Teléfono: 347 38 11
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IDENTIFICACION

C.C. ☒ NIT ☐

C.E. ☐ Otro ☐

Número: 438.019 TP 31.929

4

Número de expediente: 01.087.299 Clase: C07C 143/080

Título del Invento: **COMPOSICIONES INHIBIDORAS DE CICLOOXIGENASA-2 QUE TIENE RAPIDO ACCESO DE EFECTO TERAPEUTICO**

5

Descripción de la corrección o modificación solicitada:

De conformidad con lo previsto en el artículo 43 de la Decisión 486 de la Comisión de la Comunidad Andina, presento nuevo capítulo reivindicatorio, el cual solicito sea tenido en cuenta.

6

Comprobante de pago No. 04 - 60.314 Fecha 2 de agosto de 2004

7

Anexos:

☒ Comprobante de pago de la tasa por \$ 87.000.00

☐ Poderes, si fuere el caso

☐ Certificado de existencia y representación legal, cuando el solicitante sea persona jurídica

8

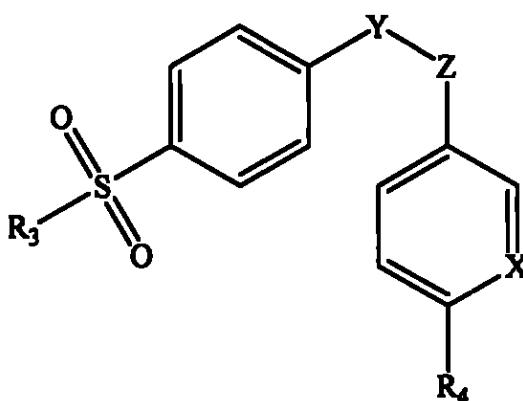
NOMBRE: EMILIO FERRERO

FIRMA: 

C.C. 438.019 de Usaquén T.P. 31.929

REIVINDICACIONES

1. Una composición farmacéutica que comprende una o más unidades de dosis suministrables oralmente, cada una de las cuales comprende, en una cantidad terapéuticamente efectiva, una droga selectiva inhibitoria de ciclooxigenasa-2 fórmula (VI):



en la cual R_3 es un grupo metilo o amino,

en la cual R_4 es hidrógeno o un grupo C1-4 alquilo o alkoxi

en la cual X es NO o CR_5 donde R_5 es hidrógeno o halógeno, y

en la cual Y y Z son independientemente átomos de carbono o nitrógeno que definen átomos adyacentes de un anillo de cinco a seis miembros que es no sustituido o sustituido en una o más posiciones con grupos oxo, halo, metilo o halometilo, donde la droga está presente en partículas sólidas con un tamaño de partícula D_{90} de alrededor de 100nm hasta alrededor de 900nm y donde el tamaño de partícula es suficiente para suministrar un C_{max} más alto y/o un T_{max} sustancialmente más corto en comparación con

otras composiciones similares en donde sustancialmente todas las partículas son mayores que 1mm.

2. La composición de la Reivindicación 1, donde las unidades de dosis están en la forma de artículos sólidos discretos.
3. La composición de la Reivindicación 2, donde los artículos sólidos son tabletas o cápsulas.
4. La composición de la Reivindicación 1 que está en la forma de una masa fluible sustancialmente homogénea desde la cual las unidades de dosis son removibles de manera medible.
5. La composición de la Reivindicación 4, donde la masa fluible sustancialmente homogénea es una suspensión líquida.
6. La composición de la Reivindicación 1, donde el anillo con 5 a 6 miembros es seleccionado a partir del grupo que consiste de ciclopentanona, furanona, metilpirazol, isoxazol y anillos de piridina sustituidos sólo en una posición.
7. La composición de cualquiera de las Reivindicaciones 1 o 2-5, donde la droga inhibitoria selectiva de ciclooxigenasa-2 es seleccionada a partir de un grupo que consiste de celecoxib, deracoxib, valdecoxib, rofecoxib, 5-5-cloro-3-(4-metilsulfonil)fenil-2-(2-metil-5-

pirinidil) piridina, 2-(3,5-difluorofenil)-3-[4-metilsulfonil]fenil]-2-ciclopenten -1-ona y (S)-6,8-dicloro-2-(trifluorometil)-2H-1-benzopiran-3-ácido carboxílico.

8. La composición de la Reivindicación 7, donde la droga inhibitoria selectiva de ciclooxigenasa-2 es celecoxib.
9. La composición de la Reivindicación 8 que comprende cerca de 10mg hasta cerca de 1000mg de celecoxib en cada unidad de dosis.
10. Un método para tratar una condición o desorden médico en un sujeto al que se le indica tratamiento con un inhibidor de ciclooxigenasa-2, el cual comprende administración de una o más unidades de dosis de una composición de cualquiera de las Reivindicaciones 1, 2-5 o 6-9 de una a aproximadamente seis veces al día.
11. El método de la Reivindicación 10 en donde la condición médica es dolor agudo.

WPC12482-101

A Mechanistic Approach to Understanding the Factors Affecting Drug Absorption: A Review of Fundamentals

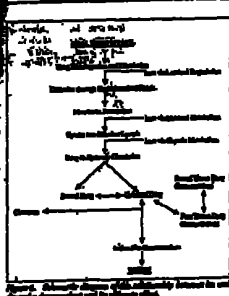
Madhup N. Madhup, PhD, and Gordon L. Amidon, PhD

The study of drug absorption is a complex task that involves understanding the interplay of various factors. This review provides a comprehensive overview of the fundamental principles governing drug absorption, from the molecular level to the clinical application. It discusses the various barriers to drug absorption, the role of transporters, and the impact of formulation and physiological factors on the overall process.

Understanding the factors affecting drug absorption is crucial for the development of effective drug formulations. This review examines the various mechanisms involved in drug absorption, including passive diffusion, active transport, and the role of the gastrointestinal tract. It also discusses the impact of formulation factors such as particle size, solubility, and release rate on drug absorption.

Absorption is the process by which a drug enters the systemic circulation. It is a complex process that involves the drug passing through various barriers, including the gastrointestinal tract, the skin, and the lungs. The rate and extent of absorption are determined by a number of factors, including the drug's physicochemical properties, the formulation, and the physiological state of the patient.

Understanding the factors affecting drug absorption is essential for the development of effective drug formulations. This review examines the various mechanisms involved in drug absorption, including passive diffusion, active transport, and the role of the gastrointestinal tract. It also discusses the impact of formulation factors such as particle size, solubility, and release rate on drug absorption.



Class II - low solubility, low permeability, high potential bioavailability

Class II drugs are characterized by low solubility and low permeability, but they have a high potential bioavailability. This is because they are often formulated as immediate-release formulations that dissolve rapidly in the gastrointestinal tract. The high potential bioavailability is due to the fact that the drug is absorbed quickly and enters the systemic circulation in a form that is readily available for use.

Class III - high solubility, low permeability, low potential bioavailability

Class III drugs are characterized by high solubility and low permeability, but they have a low potential bioavailability. This is because they are often formulated as immediate-release formulations that dissolve rapidly in the gastrointestinal tract. The low potential bioavailability is due to the fact that the drug is absorbed quickly and enters the systemic circulation in a form that is not readily available for use.

Class IV - high solubility, high permeability, high potential bioavailability

Class IV drugs are characterized by high solubility and high permeability, but they have a high potential bioavailability. This is because they are often formulated as immediate-release formulations that dissolve rapidly in the gastrointestinal tract. The high potential bioavailability is due to the fact that the drug is absorbed quickly and enters the systemic circulation in a form that is readily available for use.

where μ is the mean and σ^2 the variance. The
of finding a suitable distribution is
where μ is the mean and σ^2 the variance. The
of finding a suitable distribution is

[illegible][illegible]

CONCLUSION

Japanese industry can be estimated by determining the status of drugs in position from rigid to aqueous emulsions. This positioning behavior is often helpful information parallel to the actual effectiveness.

Indication. In general, federal drugs tend to exhibit the same response capability than their federal counterpart. Consequently, the rate of active substitution for system users can be positively affected by the use of the system.

To illustrate the effects of the use of the system, we use an example of the use of the system. The example is as follows:

Weekly tests began today to learn drug elimination rates at higher pH (where most drug action is in the un-ionized form), whereas weekly tests during chronic treatment at higher pH confirm rates drug action in the ionized form. Examples of the relationship between the percentage of drug in the un-ionized form as a function of drug pH and pK are shown in Figures 2 and 3. For this reason, by increasing the percentage of drug ionized by the un-ionized solution of 7 weeks, we can easily and decrease the elimination of 7 weeks. For example, we obtain 50% on baseline (pH 6.5) and 1.5 and 1.0 are compared to percentage when actually pH is elevated to 7.5 and 8.0, respectively. The relationship between

to ADP model, which in the vector model represents "Chomsky's" state used can increase the discriminability of "weak" odd by increasing the proportion of long-term in the initial state, thereby making it more "stable."

[illegible]

A compound's aqueous solubility, as measured by its propensity to dissolve both in pure, distilled water and in solutions with varying concentrations of hydrogen bonds with the water molecules. Generally, aqueous solubility is directly proportional to the number of hydrogen bonds that can be formed with water. As discussed later,



while very high serum activity is beneficial for drug distribution in various tissues, there were some compounds whose activity was diminished due to their

Highly volatile and your headquarters.

Although the volatility of the market is often used to describe long volatility there is some evidence that volatility may have been dominated by the unexpected growth energy response. The volatility of the energy component can be described in terms of the energy component in the aggregate shocks that the economy is exposed to. In the aggregate shocks that the economy is exposed to, the volatility of the energy component can be described in terms of the energy component in the aggregate shocks that the economy is exposed to. In the aggregate shocks that the economy is exposed to, the volatility of the energy component can be described in terms of the energy component in the aggregate shocks that the economy is exposed to.

When comparing the present chemical results to the biological water quality methods, the present chemical results for nitrate, phosphate, and the present chemical results for the number of hydrogen ions (pH), it was noted that high level of negative results.

the same characteristic as the more basic characteristic. Consequently a large degree of water vaporization in the relationship between permeability and log P is to be noted that highly permeable compounds tend to have satisfying permeation values almost identical to that of hydrophobic substances (satisfactory permeation values of 1000 ml/m²). Accordingly, hydrophobic compounds exhibit values rather than physicochemical relationships² may serve as the best predictors of a compound's membrane permeability.

[illegible]

15. The following guidelines for the correct classification of relationships are important to recognize the various patterns of events that occur during the process of drug distribution. Following treatment with lipid Wipes, such as those emulsions which have been demonstrated to be very effective in the treatment of patients with severe hypocalcemia, the following guidelines are suggested for the classification of the Wipes into the following categories:

1. The upper mandible and tongue lie against the upper incisors and the incisors with the mandibular and premolars.
2. The lower incisors have deciduous mandibles, density of all four regions increases the upper incisors, premolars and the rest of the teeth and covers the greatest amount to achieve sufficient time to 20 months.
3. The third region contains the highest density of mandibles. This region covers the majority of the mandibles, particularly in the upper incisors, premolars and the rest of the teeth and covers the greatest amount to achieve sufficient time to 20 months.
4. The fourth region is the most deciduous region of the mandibles, covering the majority of the mandibles, particularly in the upper incisors, premolars and the rest of the teeth and covers the greatest amount to achieve sufficient time to 20 months.

Conspicuously, however, it is pointed out that any possibility is not simply two-day process of mobilization and diffusion but rather represents

circle" is now, many of these persons who are
 benefited by the conditions of their state will
 very soon be in the same position as the
 preceding. The state is now in a position
 and the state is now in a position to
 in the state.

It should be remembered that the Negro-Widow
 system is a system of slavery and the
 colored people are now in a position to
 were given the same treatment as the
 colored people. The colored people are now
 in the state.

where α is the angle of incidence of the incident light in the medium with refractive index n_1 and β is the angle of refraction in the medium with refractive index n_2 . The angle of refraction β is related to the angle of incidence α by Snell's law, which states that the ratio of the sine of the angle of incidence to the sine of the angle of refraction is equal to the ratio of the refractive indices of the two media:

[illegible]

...the other hand, despite uncertainty, increasingly widespread views of the role and nature of the state in the economy have led to a growing demand for increased state intervention in the economy. This may be attributed to problems associated with the implementation of the economic reforms, the need to strengthen the state's role in the economy, and the need to strengthen the state's role in the economy.

Drug absorption. In this situation, fraction of drug absorbed can only be improved by reducing drug solubility (e.g., via the inclusion of surfactants in the product formulation). Conversely, particles also create no problem other when solubility is not a problem. In this case, a significant improvement in the fraction of drug absorbed can be achieved by increasing surface area and decreasing particle size.

On the other hand, the Massachusetts study represented the Massachusetts Department of Health's first attempt to use a community-based approach to address the health care needs of its state's residents. The study was designed to be replicable in other states and to provide a model for other states to follow. The study was designed to be replicable in other states and to provide a model for other states to follow.

[illegible]

where N_1 is the percent dissolved of the reference product, N_2 is the percent dissolved of the test product, and n is the number of wells tested.

the $\frac{1}{2}$ weight is 2.25. To obtain the sum of squares due to

7 coefficient of variation should not exceed 25% at the confidence intervals. Furthermore, it is also important to note that points 15 under all statistical conditions, the proposed reference points should be made of 25% or more. This is because, in the proposed reference points, the coefficient of variation should not exceed 25% at the confidence intervals.

SWCC has also been incorporated into and reinforced its damage-patching to make-up and post-patching damage to increase safety and maintain structural integrity. As with products that maintain Class compliance, as well as damage detection, it is possible to use types of post-patching damage may be generated in the field. It is important to note that the SWCC is not a substitute for the original design and construction of the structure.

It is by maintaining communications among these three coordinating organizations that we can effectively begin to ensure the presence of all conditions within the CI heart, the representative solution for these problems are China I or I compounds. However, to develop a KIVIC for these products, comprehensive must proceed for the changing environment in which the end of the group will be exposed as it turns down the CI track.

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CONFIDENTIALITY OF INFORMATION

An excellent example of this process is often provided by the impact that language differences in drug metabolism have on testing bioavailability. For example, the observed bioavailability differences in the oral bioavailability of theophylline in dogs, 80% in one and 100% in another,

fast-food restaurants (approximately 10% in total, or 12% in the fast-food restaurants) and 27% in shops.²² In some subjects, the

Life of a man provides an excellent discussion of the tree that can show the elements and branching reveals the similarity of compounds. It describes the organic chemistry [C] in detail.

where Q is hepatic blood flow, F is the hepatic extraction ratio, f_u is the unbound fraction of drug in the blood, and CL_{int} is the hepatic clearance (a measure of V_{max}/K_m).

When $Q \gg f_u \cdot CL_{int}$, then $CL_{hep} \approx Q \cdot f_u \cdot CL_{int} / (CL_{int} + f_u \cdot CL_{int})$, which simplifies to:

$\frac{d}{dt} C_{2r} = 0$
 $\frac{d}{dt} C_{2r}$
 Assuming that $C_{2r} \ll C_{2r}$, then $C_{2r} = C_{2r}$
 For high C_{2r} , C_{2r} is said to be linear. For
 high C_{2r} , C_{2r} is said to be linear. For
 high C_{2r} , C_{2r} is said to be linear. For

As examples of these relationships, it can be seen with the following between diffusivity (and/or interstitial concentration) and interatomic (and substitutional) $\frac{D}{D_0}$ ratio. Interstitials: high diffusion rates (e.g., $\frac{D}{D_0} = 100-1000$ at 1000 K) except for $\frac{D}{D_0} = 10-100$ at 1000 K).

infarcts, and subacute infarcts, the persistence of hepatomegaly. As expected, about 75% showed systematic blood flow to the liver, hepatomegaly has been

abstinence only drug abstinence rates in an increase in abstinence likelihood from about 30% in the control group to 50% in the intervention group. Clearly, the difference is attributable to drug-use motivation. However, the question is whether differences between intervention versus control results were attributable to the effect of interventions on the drug-use motivation, or not.

gender hypothesis: dog howls when the dog is sexually aroused

the description can be made to the following effect: The description of the invention is as follows: The invention is a method of determining the temperature of a body by measuring the rate of change of the resistance of a thermistor. The method is based on the fact that the resistance of a thermistor varies with temperature. The method is described in detail in the following paragraphs.

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[illegible][illegible]

The acidic pH generated by the brush-border Na^+/H^+ exchanger serves as the driving force for transmembrane absorption of digestives, tripeptides, and peptides (3). However, while penetration of the apical membrane appears to depend on an apical $\text{Cl}^-/\text{HCO}_3^-$ transporter (4,5,7), after crossing the membrane, these proteins that are retained in lysosomes by lysosomal phosphatases are transported across the basolateral mem-

has Communist control group or group capable of independent action.

g. Highest affinity is associated with molecules that contain
no hydroxyl groups.
a. G-bonded water and in the L configuration.

Compounds showed via the activity of the specific trans-
porters with the specific channels. Corresponding sub-
strates indicated permeability in 3 cases generally in the
order of the two following experiments¹²

ATP-Binding Cassette Transporter Protein

Another family of proteins whose impact on drug disposition is currently under intense investigation is the ATP-binding cassette (ABC) transporters. Based on numerous sequences, these are estimated to be expressed in all ABC transporters of various isoforms. Members of these genes have been associated with the development of drug resistance in various organisms.

where T_m is the melting temperature, C_0 is the initial wall concentration, K_1 is the rate constant of absorption of $1/2$ lb. P_0 is the permeability determined by the counter-current method, and P is the instantaneous permeability attributable to polymer diffusion.

Amplification of AHC sequences for the multi-enzyme digestion protocol (Fig. 1). The AHC-associated gene family consists of seven members: MEPI, MEPII, MEPIII, MEPIV, MEPIV, MEPIV, MEPIV, and MEPIV. The names are given to the genes in the order of their discovery. The names MEPI, MEPII, MEPIII, MEPIV, MEPIV, MEPIV, and MEPIV are used to refer to the genes in the order of their discovery. The names MEPI, MEPII, MEPIII, MEPIV, MEPIV, MEPIV, and MEPIV are used to refer to the genes in the order of their discovery.

[illegible]

In the past, the left's gay agenda consisted of the radical, anti-gay rhetoric. Now, even as an effective tool to the heterosexual absorption of women and children, it has become a highly effective means of controlling the variety of influences from the closeted back into the heterosexual world. It is based on the sexual exploitation of many women, including the Middle Eastern, Asian, Latin, black, gay, lesbian, transgender, and transgender, and the image and sexual liberation, and the image of the woman from the heterosexual world.

While traditional efforts to increase drug knowledge and limit demand on drug availability and profitability are recent strategies have focused on the development of programs as a strategy for changing drug consumption. By targeting broad segments of consumers such as

Fox was still-banked enough to find the one effect of its existence. Although not the only cause, dependent secondary mechanisms affecting dormancy, it appears to have the most widespread importance due to its high intertidal concentration, its location within the intertidal, and its broad and stable capacity to go to dormancy on the order of the 1000 mg/l level. In contrast, with additional or without loss of the intertidal zone, the intertidal zone, or an entire

PHARMACEUTICALS AND PHARMACEUTICS

ABC transports proteins from cytoplasm into the nucleus. This includes two large transcription factors (Jun and Fos) through which the mitogen-

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1. In relation to marine mammals, however, additional information also appears to be required by the authors of the report. The authors have indicated that they are not aware of any information on the occurrence of cetaceans in the waters of the United States. This is not the case. The U.S. Navy has been conducting a systematic survey of the waters of the United States for marine mammals since 1966. The results of this survey are being compiled and will be available in the near future. The authors of the report should be aware of this information and should include it in their report.

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on a understanding of the present technological state of the art, and the need for further research in the field of the development of the gas turbine engine. The author also points out the need for further research in the field of the development of the gas turbine engine. The author also points out the need for further research in the field of the development of the gas turbine engine.

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MANAGEMENT OF THE CASE

ALISTON AND ANDERSON

UNDERSTANDING THE FACTORS AFFECTING DATA ACQUISITION

1. The first factor is the nature of the data being collected. The data may be continuous or discrete, and it may be structured or unstructured. The nature of the data will determine the type of data acquisition system that is required.

2. The second factor is the volume of data being collected. The volume of data will determine the size of the data acquisition system that is required.

3. The third factor is the speed of data collection. The speed of data collection will determine the type of data acquisition system that is required.

4. The fourth factor is the accuracy of data collection. The accuracy of data collection will determine the type of data acquisition system that is required.

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7. The seventh factor is the reliability of the data acquisition system. The reliability of the data acquisition system will determine the type of data acquisition system that is required.

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10. The tenth factor is the scalability of the data acquisition system. The scalability of the data acquisition system will determine the type of data acquisition system that is required.

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WATKINS AND ALLEN

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MANUFACTURING AND TECHNOLOGY

MODELING OF INTESTINAL DRUG ABSORPTION: ROLES OF TRANSPORTERS AND METABOLIC ENZYMES (FOR THE GILLETTE REVIEW SERIES)

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Oral Drug Absorption

The absorption of drugs via the oral route is a subject of intense and continuous investigation in the pharmaceutical industry since good bioavailability implies that the drug is able to reach the systemic circulation by mouth. Oral drug absorption is affected by both drug properties and the physiology of the gastrointestinal tract (GIT), or patient properties, including drug dissolution from the dosage form, the manner in which drug interacts with the aqueous environment and membrane, permeation across membrane, and irreversible removal by first-pass organs such as the intestine, liver and lung (Martinez and Amidon, 2002). The purpose of this mini-review is to highlight the processes governing drug bioavailability when the drug is already in solution, and emphasize the roles of intestinal transporters and metabolism on oral bioavailability. The description of physical models for drug dissolution on drug absorption (Higuchi, 1967) or hepatic modeling (Pang and Chiba, 1994; Pang et al., 1998; Abu-Zaira and Pang, 2000), however, is beyond the scope of this work.

The intestine, in addition to the liver, is an important device that regulates the extent of absorption of orally administered drugs, since the intestine and liver are involved in first-pass removal (Higuchi et al., 1971; Rowland, 1972). The majority of drug absorption occurs at the small intestine because of the large surface area since the presence of villi and microvilli increases the absorptive area manyfold. The duodenum and jejunum possess the greatest surface area due to the highest concentration of villi and microvilli in these regions, and surface area is least for the ileum (Mague and Dailey 1986).

The circulation of the intestine is unique in that the intestine is the anterior or portal device that regulates the flow of substrates to the liver. The intestinal venous blood constitutes the majority of the blood supply to the liver accounting for 75% of total liver blood flow. For drugs that are highly cleared by the intestine, the contribution of the

liver or lung to drug metabolism will become reduced, whereas for drugs that are poorly extracted by the intestine, the substrate is able to reach the next device, the liver and the lung, for removal (Gugler et al., 1973; Xu et al., 1989; Hanyama et al., 1990). The concentration of drug entering the intestine (Xu et al., 1989; Hanyama and Pang, 1990) and the intestinal flow rate (Chen and Pang, 1997) alter the rate of drug delivery and affect the degree of saturability of intestinal enzymes. These ultimately affect the rates of intestinal and hepatic first-pass metabolism. Additionally variables such as other drugs or food that alter the transit times within the gastrointestinal tract further modulate the absorption of substrates by the intestine (Welling, 1984; Kikawa and Higaki, 2002).

Drug Properties

Many efforts exist to interrelate the physicochemical properties of the drug with absorption. The drug, whether a weak acid or a weak base, and its pK_a determine the extent of ionization according to the pH partition hypothesis at various pH values (pH 1.3 for stomach and 6 for intestine) of the gastrointestinal tract (Hagben et al., 1957, 1959; Schuster et al., 1957a,b). Deviations from the predictions were found, and these had been explained by the presence of an unstirred water layer (USWL) (Suzuki et al., 1970a,b), or a microclimate pH (Winn, 1977). The concept of absorption potential was then utilized to describe drug absorbability based on the partition coefficient, the solubility dose, and fraction ionized (Dressman et al., 1983; Macheras and Symlidis, 1989; Yu et al., 1996). Drugs that are un-ionized or that undergo hydrogen bonding exhibit a much greater lipophilicity toward membrane permeation than their ionic counterparts. Too many hydrogen donor or acceptor groups, however, is not good (Lipinski et al., 1997). Lipophilicity, a major determinant for predicting the extent of membrane permeation, is often correlated with the partition coefficient, when aqueous solubility is not exceeded and when the unstirred water layer is not an imposing barrier (Ungell et al., 1998). For very lipophilic agents, absorption may be rate-limited by the inability of drug traversing the USWL. For very hydrophilic or polar agents, the converse is true, such that membrane resistance is higher than the aqueous layer resistance. However, when drugs possess both hydrophilic and lipophilic qualities and permeate the USWL and membrane well, blood perfusion rate becomes the overall rate-limiting step for absorption. Predictive models based on Lipinski's Rule of Five (Lipinski et al., 1997), the quantitative structure-bioavailability relationship (Andrews et al., 2000), or other quantitative structure-activity relationship models (Nortander et al., 1999; Zhao et al., 2001, 2002; Klopman et al., 2002) have been developed to forecast the drug permeation potentials. The Rule of Five predicts that oral availability will be poor when there are more than 5 H-bond donors, 10 H-bond

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Abbreviations used are: GIT, gastrointestinal tract; USWL, unstirred water layer; Pgp, P-glycoprotein; MDR1, multidrug resistance gene product 1; MRP2, multidrug resistance-associated protein 2; BCRP, breast cancer resistance protein; CAT, compartmental absorption and transit; ACAT, advanced CAT; Mxt1, monocarboxylic acid transporter 1; Oatp3, organic anion-transporting polypeptide 3; UGT, UDP-glucuronosyltransferase; PGT, sulfotransferase; GST, glutathione S-transferase; MPT, mean residence time; P450, cytochrome P450; TM, traditional model; SFM, segregated flow model; STM, segmented, traditional model; SSFM, segmented segregated flow model.

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acceptors, the logP value is greater than 5, and the molecular weight exceeds 500 (Lipinski et al., 1997).

Drug permeation across the intestinal membrane is described by the effective permeability coefficient, P_{eff} , or the overall rate of loss of compound per unit area (Hillott et al., 1980; Amidon et al., 1981; Ho et al., 1983; Fagerholm et al., 1996). The coefficient P_{eff} may be estimated from luminal perfusion studies by measurement of drug loss across the lumen of any given segment, where $C_{out,lumen}$ and $C_{in,lumen}$ are the concentrations of drug leaving and entering the lumen, respectively; r is the radius, l is the length, and Q_{lumen} is the experimentally determined luminal flow rate.

$$P_{eff} = \frac{Q_{lumen}}{2\pi r l} \ln \left(\frac{C_{out,lumen}}{C_{in,lumen}} \right) \quad (1)$$

Since changes in water content in the lumen occur for these studies, the change in water volume may be corrected for by the inclusion of a nonabsorbable substance such as polyethylene glycol 4000. For passively absorbed drugs, P_{eff} is dependent on the physicochemical properties of the substrate, including lipophilicity, molecular size, hydrogen bonding capacity, and polar surface area (Whitwater et al., 1998), and is related to the permeability in the aqueous unstirred water layer (P_m) and the permeability across the membrane (P_{ms}), and may be estimated from the following equation (Ho et al., 1983).

$$\frac{1}{P_{eff}} = \frac{1}{P_m} + \frac{1}{P_{ms}} \quad (2)$$

The fraction of dose absorbed (F_a or $C_{out,lumen}/C_{in,lumen}$) is estimated as that portion of the dose which disappeared from the intestinal lumen. The fraction of dose absorbed will depend on the physicochemical properties of the compound, the presence of carrier-mediated systems for absorption and excretion, and intestinal metabolism within the enterocytes.

The Intestine, a Drug Metabolizing and Excretion Tissue

The intestine is noted for its absorptive function because of the presence of villi and microvilli and transporters for organic anions and cations (Fel et al., 1994; Sadee et al., 1995; Truji and Tamei, 1996; Arimori and Nakano, 1998; Craddock et al., 1998; Koepsell 1998; Ho et al., 1998; Zhang et al., 1998). Additionally, there exist drug-metabolizing enzymes for oxidation due to the appreciable cytochrome P450 3A (CYP3A4 in humans) (Hosonouchi et al., 1975; Watkins et al., 1987; Dubey and Singh, 1988a; Kolacz et al., 1992; Thummler et al., 1996; Paine et al., 1996, 1997) and conjugation enzymes (Dubey and Singh, 1988b; Ilett et al., 1990), and efflux transporters at the apical and basolateral membranes (Lin et al., 1999; Sumida and Sugiyama, 2000; Wachter et al., 2001) (Fig. 1). Drug excretion occurs at the villous tips of the enterocytes at the apical membrane via the 170-kDa P-glycoprotein [Pgp; or the multidrug resistance gene product (MDR1)] (Thiebaut et al., 1987; Haing et al., 1992; Smit et al., 1998a,b) and multidrug resistance-associated protein 2 (MRP2) (Paulusma et al., 1996; Gotch et al., 2000; Motino et al., 2000) that cause drug efflux into the lumen, effectively reducing the sojourn of drug within the enterocyte (Salich et al., 1996; Lowy et al., 1997b; Kim et al., 1998). Many drugs are substrates of both Pgp and cytochrome P450 3A (Torne et al., 1996; Wachter et al., 1998; Benet and Cousins, 2001). These are exemplified by verapamil (Salich and Amstutz, 1995; Sandström et al., 1998); anticancer drugs such as vincristine, etoposide, daunorubicin, and paclitaxel (Lee and Huang, 1995; Sonnichsen et al., 1995; Nakayama et al., 2000; Chico et al., 2001; Wachter et al., 2001; Abraham et al., 2002); digoxin (Cavet et al., 1996; Greiner et al., 1999); indinavir, the human immunodeficiency

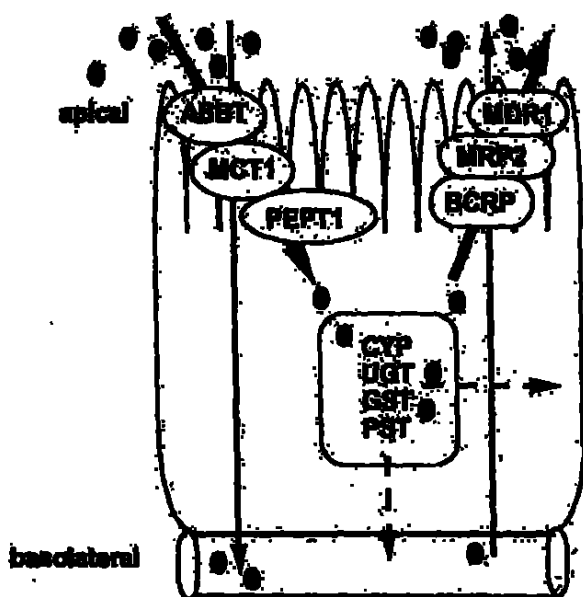


Fig. 1. Schematic diagram of the enterocyte of the intestine showing absorptive and efflux transporters at the apical and basolateral membranes, and enzymes for intracellular metabolism.

virus protease inhibitor (Hochman et al., 2000, 2001; Li et al., 2002); and immunosuppressive agents cyclosporin (Gan et al., 1996; Lowy et al., 1997b), tacrolimus (Lampen et al., 1995; Hashimoto et al., 1998; Hashida et al., 2001), and sirolimus (Lampen et al., 1998; Paine et al., 2002).

Gastric Emptying and Intestinal Motility

The rate of gastric emptying strongly impacts the rate and extent of intestinal drug metabolism and drug absorption. Various disease conditions and food intake affect stomach emptying and/or intestinal transit (Welling, 1984). Double peak absorption has been correlated with altered gastric motility (Oberle and Amidon, 1987; Pinaud et al., 1987; Suttle et al., 1992; Langguth et al., 1994; Lipka et al., 1995; Suttle and Brouwer, 1995; Wang et al., 1999; Takamatsu et al., 2002; Kimura and Higaki, 2002; Yin et al., 2003) as well as other factors including the presence of adjuvants (Bae et al., 2002) or bile salts (Lennernäs and Regardt, 1993). The data showing double peaks during absorption have been modeled as the discontinuous oral absorption model (Witcher and Boudriot, 1996). Invariably, a delay in stomach emptying reduces the rate of drug absorption since the rate of delivery to the site of absorption, the small intestine, is prolonged (Table 1). With regard to gastric emptying, drugs may be classified as

TABLE 1
Effect of delayed gastric emptying and effect of metabolism and excretion on drug absorption

Drug Class	Rate of Absorption	Extent of Absorption
Acid-labile compounds	↓	↓
Relatively insoluble compounds	↓	↓
Compounds with good water and lipid solubility	↓	↔
Compounds whose absorption is mediated by intestinal transporters	↓	↑
Compounds that are metabolized by intestine	↓	↓
Compounds that are effluxed by intestine	↓	↓

TABLE 2
Involvement of intestinal transporters for drug absorption (see also references contained in Tugli and Tamai, 1999)

Transporter	Substrate	Reference
slat or flat Naal sodium-dependent bile acid transporter	Taurolithate	Schneider et al. 1993; Cuddihy et al., 1998
Pept1 Oligopeptide transporter 1	Anticoagulants, β -amino lactam antibiotics (acyclovir, cephalexin, cephazolin), acetylcholinesterase inhibitors (donepezil, galantamine), benzothiazine dihydrochloride, Lactase and pyruvate transport (simple oral and alkyl salts)	Pol et al., 1994
Mot1 Monocarboxylic acid transporter 1	Taurolithate, oxalate salts	Walters et al., 2000
Oatp3 Intestinal organic anion transporting polypeptide 3		
NPT1 Sodium-dependent phosphate transporter 1	Furosemide, furosemide	Tugli and Tamai, 1989
OCT1 Organic cation transporter 1	Sparidone, sparteine	
Pelle solid transporter	Methotrexate	
CNT Nucleoside transporters NT1 (purine) and NT2 (pyrimidine)	Zidovudine, stavudine, didanosine, ribavirin, allopurinol, 5-fluorouracil	
Amino acid carrier	D- and L-tryptophan, L-phenylalanine, leucine, tyrosine, alanine	
MDR1 or Pgp Multidrug resistance protein 1 or P-glycoprotein	Doxorubicin, rhodamine 123, verapamil, digoxin, colchicine, vincristine, etoposide β -lactams (penicillin, pefloxacin, cefepime, ticarcillin), Griseofulvin, phenytoin, carbamazepine, valproic acid, vitamin C, vincristine	Gotch et al., 2000; Motino et al., 2000
Mpr2 Multidrug resistance-associated protein 2		
BCRP Breast cancer resistance protein	Topotecan, irinotecan, anthracyclines	Doyle et al., 1998; Zamber et al., 2003
Mpr3		Rost et al., 2002

1) solid-labile compounds, 1) relatively insoluble compounds, 3) drugs with good water and lipid solubility, and 4) drugs absorbed by carriers. For the solid-labile drugs such as penicillin and aspirin, a greater degradation occurs with prolongation of stay in the stomach, diminishing the extent of absorption (Terry et al., 1982; Ali, 1985). By contrast, increasing the transit time of relatively insoluble compounds in the stomach favors drug dissolution and improves the extent of the absorption of griseofulvin and phenytoin (Aoyagi et al., 1984; Hamaguchi et al., 1993), whereas no change in extent exists for well absorbed drugs such as acetaminophen and fentanyl, compounds of good water and lipid solubility (Terry et al., 1982; Choi et al., 1993). For drugs whose transport into the intestine is via apical transporters, however, it is envisaged that the reduced intermittent release of drug from the stomach to the intestine brings about a deterioration of the transporter system, rendering an increase in the extent of drug absorption (Table 1).

Methods to Study Intestinal Transport and Metabolism

Because of the significance of the intestine as an important first-pass organ, high-throughput in vitro systems have been developed to assess the importance of intestinal absorption, metabolism, and excretion for the prediction of drug-drug interactions. Gene expression systems (Smit et al., 1998; Cvetkovic et al., 1999; Gotch et al., 2000; Shitara et al., 2002) provide direct information on the involvement of individual transporters or enzymes. Then there are the intestinal membrane segments/preparations (Wilson and Treanor, 1975; Hopfer et al., 1976; Lasker and Rickert, 1978; Johnson et al., 2001), cells (Koster and Noordhoek, 1983; Traber et al., 1991), everted sacs (Munck, 1965; Barr and Riegelman, 1970; Kaplan and Coffey, 1972), and the Ussing chamber (Fiddian-Green and Silen, 1975; Rogers et al., 1987; Lampen et al., 1995). For flux measurements, a donor

compartment is used for drug administration and a receiving compartment is used for sampling. With drug given to the mucosal side, sampling allows the examination of drug absorption, metabolism, and efflux as well as entry into the basolateral compartment. Moreover, drug may be given at the serosal compartment to ascertain the net flux from the basolateral side to the mucosal lumen. Drug efflux and excretion has been studied with vesicles prepared from the basolateral and apical membranes (Weisberg et al., 1986; Tugli and Tamai, 1989; Bair et al., 1991).

A popular in vitro system is the Caco-2 cell line, derived from human colon carcinoma cells (Hidalgo et al., 1989; Hunter et al., 1990), that contains the Pgp (Hunter et al., 1993a,b). A slight drawback may be the existence of a USWL that may pose as a barrier for lipophilic drug transport (Hidalgo et al., 1991). The development of the Caco-2 cell has greatly facilitated progress and led to the testing of diverse drug classes as Pgp substrates (Barton et al., 1993; Hooys et al., 1996; Tanaka et al., 1996; Teraso et al., 1996). The differentiated Caco-2 cell monolayers were initially used to study drug efflux by the Pgp, or the multidrug resistance gene product MDR1. Involvement of Pgp is inferred when the basolateral to apical flux (B to A) exceeds that of A to B. It was further found that, upon culture in 1 α -25-dihydroxy vitamin D₃ for 2 weeks postconfluence, cytochrome P450 3A activities were up-regulated (Cimmi et al., 1996, 2000; Schmidlin-Ren et al., 1997; Thummler et al., 2001). More recent development involved transfection with the cytochrome P450 gene and stimulation by butyrate (Cummins et al., 2001) to provide the added P450 activity. The incubation system, the donor and receiving compartments separated by the cell monolayer, is an efficient, high-throughput system for examination of whether newly developed pharmaceuticals are substrates of cytochrome P450 3A4 and/or P-glycoprotein such that interactions with other drugs may be predicted.

TABLE 3

Segmental distribution of transporters and enzymes among the intestinal segments

Transporter	Segmental Distribution	References
Astt Pept1	distal intestine > proximal intestine proximal intestine > distal ileum function: (jejunum > ileum > duodenum) duodenum < jejunum > ileum	Schneider et al., 1993; Akmal et al., 1996 Pei et al., 1994
Mst1 Oatp3 CNT CYP3A UGT PST GST	highest in jejunum highest in jejunum duodenum ~ jejunum > ileum duodenum ~ jejunum > ileum duodenum ~ jejunum > ileum duodenum ~ jejunum > ileum	Tunai et al., 1999; Cong et al., 2001 Wahara et al., 2000 Ngo et al., 2001 Faine et al., 1996, 1997; Li et al., 2002 Kuster et al., 1985 Schwartz and Schwenk, 1974 Fukui et al., 1977
Entero cellinase	proximal > distal	Hajjebauer et al., 1984
Myp2 Mdr1 Myp3 (uncoupled)	duodenum ~ jejunum > ileum jejunum ~ ileum > duodenum ileum > jejunum > duodenum	Motilko et al., 2000; Gosh et al., 2000 Bilich and Aung, 1995; Collett et al., 1999; Li et al., 2002 Rost et al., 2002

TABLE 4

Route-dependent intestinal metabolism

Drugs	Route of Administration		References
	Intestinal	Systemic	
Acetaminophen	Glucuronide/Sulfate	ND	Pang et al., 1986
Enoxapril	Enalaprilat	ND	Pang et al., 1985
Cyclosporin	+	ND	Dokshorn et al., 1995
Theophylline	+	ND	Lampen et al., 1993
Midazolam	1-Hydroxymidazolam	low	Faine et al., 1996; Thummler et al., 1996
(-)-Amphetamine	(-)-Cacbovir	low	Wen et al., 1999
Morphine	Morphine 3β-o-glucuronide	ND	Doherty et al., 2000

The *in situ* vascularly perfused rat small intestine preparation is a useful preparation for studying the disposition of both orally and systemically administered agents (Windmuller and Speth, 1977, 1981; Pang et al., 1986; Hirayama et al., 1989; Doherty and Pang, 2000). In this preparation, the native architecture of the small intestine is maintained with respect to the circulation such that the extents of metabolism, absorption, and secretion can be studied simultaneously (Pang et al., 1985; Xu et al., 1989; Hirayama and Pang, 1990). The technique allows for single-pass or recirculating experiments involving systemic or luminal drug administration, including luminal administration in closed loops or segments (Pang et al., 1986; Cong et al., 2001). In other instances, the intestine-liver preparation may be used for the study of first-pass metabolism and for examination of the role of the intestine on regulation of hepatic metabolism (Pang et al., 1985; Chen and Pang, 1997).

In vivo techniques exist for the study of intestinal drug absorption. The Doherty method entails use of an *in situ* rat gut technique for drug administration into the lumen (Doherty et al., 1989; Shin and Beck, 1988). In some rat preparations, the inflow and outflow of a select segment were monitored for drug disappearance, and arterial blood was sampled and the volume of blood was replenished by transfusion (Barr and Elgethun, 1970). Some studies involve luminal instillation of drug to select or closed segments of rats (duodenum, jejunum, or ileum) (Hirayama et al., 1990; Cong et al., 2001) or humans (Grunett and Richter, 1994; Grunett, 1996; Grunett et al., 1994, 1996). Gene knockout mice (Kim et al., 1998; Smit et al., 1998; Groiner et al., 1999) and mutant animal models (Gosh et al., 2000) have also been utilized to examine intestinal transport. The surgical manipulation of portacaval transposition allowed the direct assessment of systemic intestinal removal in the absence of the liver (Gugler et al., 1975; Bilezikian et al., 1982; Lo et al., 1982). The method is almost analogous to the oral and systemic administrations of the test

drug, midazolam, to anhepatic patients for the estimation of intestinal drug metabolism (Faine et al., 1996). Through varying sites of drug administration, namely, orally, intraperitoneally, and intravenously, comparison of the area under the curves under first-order conditions yields the available fraction of the intestine and liver (Mistry and Houston, 1985, 1987; Hirayama et al., 1990).

Transporters for Absorption And Efflux at Apical Membranes

Much attention is given to the presence of transporters at the apical or brush-border membrane of the intestine, not only for drug absorption but also for excretion (Table 2). The various apical transporters for organic anions and cations have been reviewed (Tajiri and Tunai, 1996; Koopell, 1998; Zhang et al., 1998). The ABC (ATP-binding cassette proteins) efflux transporters, Pgp for lipophilic entities and MRP2 for drug conjugates, are both present on the intestinal membrane. Moreover, the newly characterized breast cancer resistance protein (BCRP) multidrug transporter confers resistance to mitoxantrone, topotecan, the anthracyclines, and related drugs in cell lines (Doyle et al., 1998; Allen et al., 1999, 2002; Miyake et al., 1999; Jonker et al., 2000; Zamber et al., 2003). These efflux transporters are known to deflect drug absorption.

Heterogeneity of Intestinal Transporters. Analogous to that found for the liver, there exists an increasing body of literature on the heterogeneity of intestinal transporters (Table 3). The absorption of salicylate (Ungell et al., 1998), antipyrine (Raoof et al., 1998), acetaminophen (Grunett and Richter, 1994), griseofulvin (Grunett, 1996), and (-)-cacbovir (Boris and Zimmerman, 1994) was found to be the same among all segments. Metoprolol absorption is the same among segments in humans (Vidon et al., 1985). Preferential absorption in segmental regions has also been noted for the intestinal absorption of nitidine (Stille and Brouwer, 1995) and diltiazem (Honey et al., 1995). In like fashion, the absorption of ranitidine

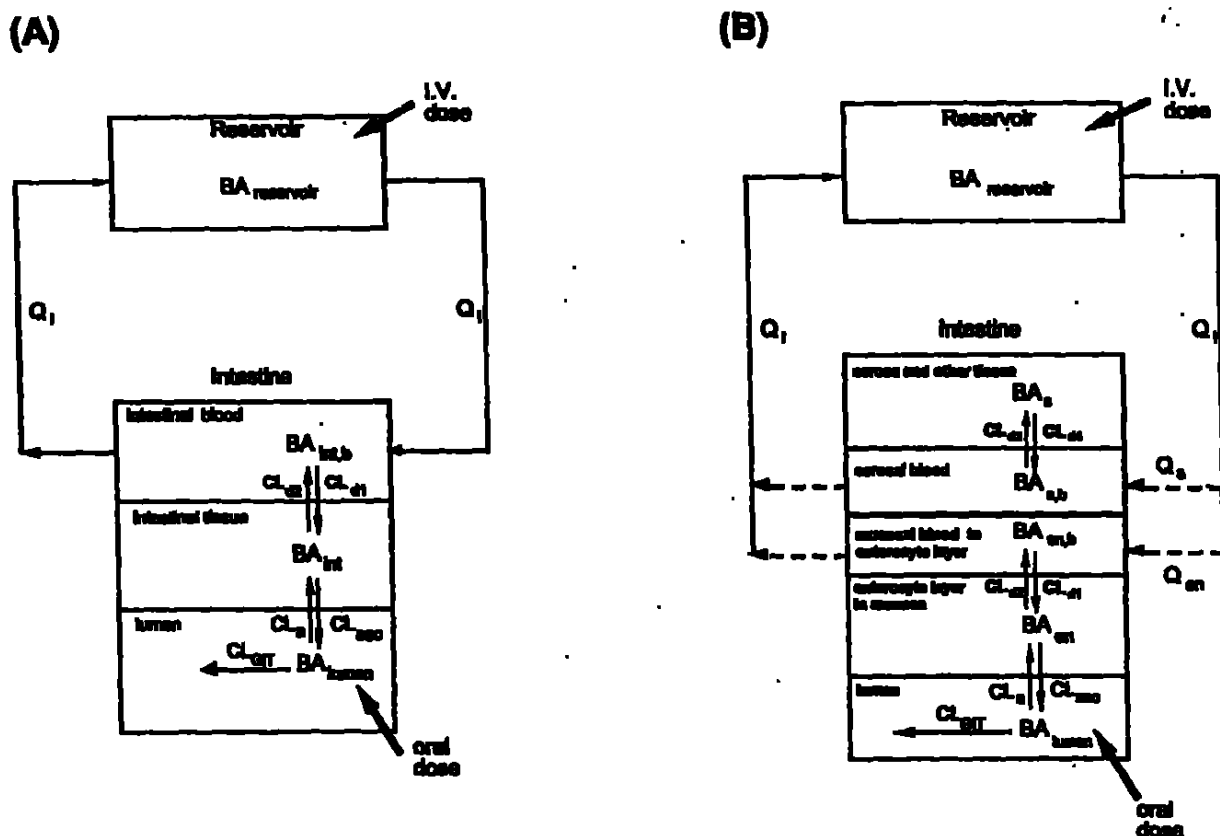


FIG. 2. Flow of drug and metabolite in the TM (A) and IFM (B).

Note that the entire oral dose passes through the enterocyte region for TM and IFM, whereas only partial intravenous dose reaches the enterocyte region for the IFM. For TM, the intestinal blood (Q_1) perfuses the entire intestinal tissue, the site of metabolism and absorption from the lumen. For IFM, intestinal blood is segregated to perfuse the nonmetabolizing (Q_2) and enterocyte-mucosal (Q_3) regions. Drug equilibrium with these is the corresponding tissue layers with intracellular transfer clearances CL_{int} and CL_{hep} for TM or CL_{int} and CL_{hep} and CL_{gut} for IFM. The absorptive, metabolic, and efflux activities within the villus tips of the mucosal layer are represented by the rate constant, k_a , and metabolic and secretory intracellular clearances, CL_{int} and CL_{hep} , respectively. Gastrointestinal transit clearance is denoted by CL_{gut} . Adapted from Cong et al. (2000), with permission.

(Gronnet et al., 1994) and talinolol (Gronnet et al., 1996) is higher in the proximal intestine in humans. For hydrochloride, atenolol, furosemide, and cimetidine (Lemmens, 1998), the net mucosal to serosal absorption was greater for the jejunum than for the ileum. For verapamil (Hunter et al., 1990), phenytoin, albuterol, gentamicin, metoprolol, omeprazole, propranolol, fentanyl, erythritol, dDAP (Ungell et al., 1998), and etoposide (Makhey et al., 1998), net mucosal to serosal absorption was greater for the ileum over the jejunum.

Recent advances in expression cloning of intestinal transporters have provided more definitive tools for the examination of regional distribution of the transporters (Fei et al., 1994; Schneider et al., 1995; Mottino et al., 2000; Walters et al., 2000; Ngo et al., 2001). There are consequences of heterogeneously distributed apical absorptive and secretory transporters. Varying rate constants for benzoic acid intestinal uptake were observed among the segments, and a higher absorption rate constant was shown for the jejunal segment versus the duodenal or ileal segment in the perfused rat intestine preparation. The higher jejunal distribution of Mct1 was confirmed with Western blotting along the length of the rat small intestine (Cong et al., 2001). The proton-coupled oligopeptide transporter of the rabbit was found more abundantly in the proximal intestine (duodenum and jejunum) (Fei et al., 1994); the net organic anion transporter 3, Oatp3, is higher

in the jejunum (Walters et al., 2000); and the apical bile salt transporter (abst) predominates in the distal ileum of the hamster and rat (Wong et al., 1994; Schneider et al., 1995). Gatch et al. (2000) demonstrated the greater mRNA expression of MRP2 in the rat jejunum, followed by the duodenum and ileum, with very little in the colon, as confirmed by Mottino et al. (2000). The secretion of the glutathione conjugate 2,4-dinitrophenyl-S-glutathione by MRP2 was greatest in the jejunum, as expected by mRNA expression (Gatch et al., 2000). The efflux transporter rat MRP2 is higher in the proximal region (Gatch et al., 2000; Mottino et al., 2000), whereas the P-gp efflux pump is higher distally at the jejunum and ileum (Thiebaut et al., 1987; Hunter et al., 1990; Chimalzo et al., 1995; Lown et al., 1997b; Collett et al., 1999; Nakayama et al., 2000; Stephens et al., 2001; Li et al., 2002). The rat basolateral MRP3 that transports drug out of the cell is higher toward the distal ileum and colon (Rost et al., 2002). The nonhomogeneous distributions of the transporters (Table 3) are expected to affect drug absorption and bioavailability.

Heterogeneity of Intestinal Enzymes for Metabolism. The intestinal tissue is endowed with phase I and II enzymes, although at lower levels than those for the liver. Drug metabolizing enzymes: UDP-glucuronosyltransferases (UGTs), sulfotransferases (SFTs), and glutathione S-transferases (GSTs) exhibit a decreasing gradient along the

intestinal wall, from duodenum to ileum (Clifton and Kaplowitz, 1977; Piekus et al., 1977; Schwarz and Schwank, 1984; Dubey and Singh, 1988a). The human intestinal CYP3A4 shows a slightly lower level at the duodenum before levels rise again at the jejunum, then finally decreasing toward the ileum (Paine et al., 1996; Thummler et al., 1996). The same was found in animals (Hosnuch et al., 1975; Li et al., 2002).

Route-Dependent Intestinal Metabolism/Excretion

Route-dependent intestinal metabolism has been observed (Table 4). "Route-dependent" intestinal metabolism describes a greater intestinal metabolism/extraction of drug upon oral or luminal dosing versus "systemic" dosing. In studies pertaining to the perfused, rat small intestine preparations, greater extents of metabolism were noted for acetaminophen (Pang et al., 1986), analapril (Pang et al., 1985) morphine (Doherty and Pang, 2000) and (-)-amoxiclovir, the prodrug that was converted to (-)-carbovir (Wen et al., 1999), when given luminally, whereas metabolism was either absent or negligible when the drug dose was given into the reservoir for systemic delivery. These results from the vascular intestinal perfusion model mirrored the observations on midazolam hydroxylation in humans. The drug exhibits a low intestinal extraction ratio (0.09) in asheptic patients undergoing liver transplantation with systemic administration, but extensive first-pass metabolism was noted orally (extraction ratio of 0.43), with much of the intestinally formed primary metabolite, 1-hydroxy midazolam, reaching the hepatic portal blood (Paine et al., 1996; Thummler et al., 1996, 1997). Interestingly, intestinal CYP3A4 levels in humans correlated well with rates of midazolam 1'- and 4-hydroxylation (Thummler et al., 1996) but failed to show a relation with the erythromycin breath test (Lowin et al., 1994, 1997b) that is ordinarily used to correlate with liver CYP3A4 levels (Wachter et al., 1989). The sensitivity of the intravenous erythromycin test to liver function only translates to the inaccessibility of the systemically administered substrate to the intestinal mucosal (CYP3A4) enzymes. The overall findings infer that the enzymes for presorptive intestinal metabolism are present on enterocytes facing the lumen and are unavailable to drugs in the circulation. A general hypothesis was put forth to explain route-dependent intestinal metabolism: intestinal drug

metabolism behaves as if it were a presorptive event occurring predominantly during absorption, but little or no intestinal removal occurs for drug in the systemic circulation due to the inaccessibility of enzymes (Doherty and Pang, 1997). Analogously, pretreatment of humans with rifampin, a pregnane X receptor ligand, on Pgp secretion exerted an effect only on the oral but not the intravenous kinetics of digoxin (Greiner et al., 1999), suggesting that the intestinal Pgp is accessible for digoxin administered into the lumen.

Intestinal Modeling

The dynamic interactions of metabolism and secretion, and the role of transporters on drug absorption have been under considerable debate. The thorough understanding of intestinal processes would be learnt through modeling of intestinal data only in the absence of the contribution from the liver. Recent studies on the interactions between the P450s and Pgp in vitro have led to the conclusion that intestinal metabolism is enhanced by the secretory action of P-glycoprotein due to an increase in the mean residence time (MRT) of drug in the intestine (Benet and Cummins, 2001; Cummins et al., 2001; Johnson et al., 2001). Theoretical examinations on Caco-2 cells and intestinal vascular perfusion systems support the notion that the mean residence time was increased, but metabolism under linear conditions was in fact decreased in the presence of secretion, even though the mean residence time has increased; under nonlinear metabolism, instances exist whereby the rate of metabolite accrual may increase, even though the ultimate amount of metabolite formed remained equal to the dose (Tam et al., 2003a). However, under cases of nonlinear metabolism, increased secretion together with rapid re-entry of drug from the apical compartment to the cell evokes increased rates of drug metabolism. The reason for this anomaly is attributed to the destruction of intestinal enzymes (Tam et al., 2003a).

The next question is, what about the intact intestine? How should data arising from studies pertaining to intestinal metabolism, efflux, absorption, and gastric emptying/intestinal transit be handled? Modeling efforts have included the gastrointestinal absorption and kinetic models that examine absorption of drug from the stomach, duodenum, upper and lower jejunum and ileum, cecum, and large intestine with

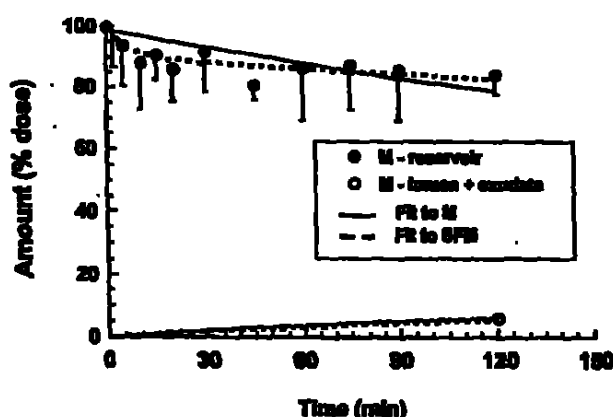


FIG. 3. Fits of tracer $[^3\text{H}]$ morphine data from the vascularly perfused, recirculating rat small intestine preparations to the TM and SPM, after systemic administration of dose into the reservoir (data from Cong et al., 2003).

There was a total lack of morphine glucuronide formed. Only morphine was converted into the lumen (and luminal fluid that was collected as exudate). Note the superior fit of the data to the SPM. Adapted from Cong et al. (2003), with permission.

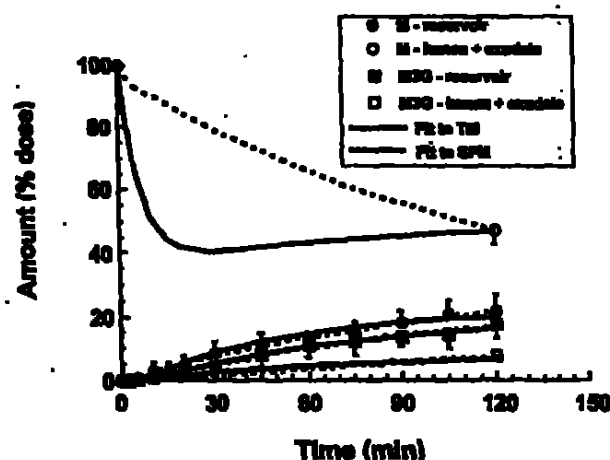


FIG. 4. Fits of tracer $[^3\text{H}]$ morphine data from the vascularly perfused, recirculating rat small intestine preparations to the TM and SPM, after duodenal administration of dose into the intestinal lumen (data from Cong et al., 2003).

Note that morphine glucuronide was formed with luminal administration of morphine. Both morphine and morphine glucuronide were found in the lumen and luminal fluids that were collected as exudate. Note the superior fit of the data to the SPM. Adapted from Cong et al. (2003), with permission.

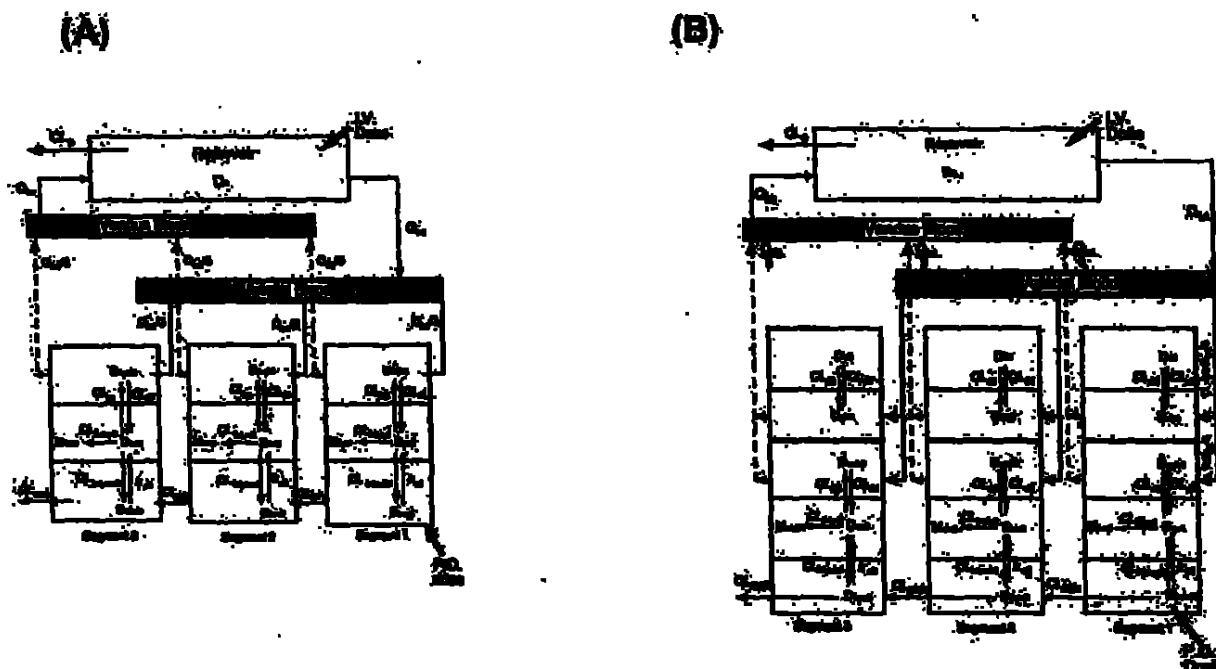


Fig. 5. The SFM, which views the intestine as three equal segments (or expanded i^{th} compartments where $i = 1, 2, \text{ or } 3$) of TM (Fig. 2A) with metabolism taking place to form the metabolite (M) in the intestinal tissue (A), and the SFM, an expansion of the SFM (Fig. 2B), which views the intestine as three segments (or expanded i^{th} compartments where $i = 1, 2, \text{ or } 3$) with low and partial flow to the enterocyte regions ($f_e Q_{e,i}$), where f_e is the fraction of total intestinal flow ($Q_{e,i}$) perfusing the enterocyte region, where the entrance and efflux transporter reside and bulk flow $[(1 - f_e) Q_{e,i}]$ in the "other" nonabsorbing region (B). Drug (D) in blood equilibrates with that in the corresponding tissue layer with intrinsic transfer clearances CL_{in} and CL_{out} for both SFM and TM. The absorptive, metabolic, and efflux activities within the villus tips of the mucosal layer are represented by the rate constant, k_a , and metabolic and secondary intrinsic clearances, CL_{met} and CL_{sec} , respectively, for each i^{th} segment. The gastrointestinal transit clearance is denoted by CL_{trans} . Adapted from Tam et al. (2003b), with permission.

varying transit times and absorption (Kikawa and Higaki, 2002). There had been theoretical investigations on the compartmental absorption and transit (CAT) model that considered the transit and absorption of drug in the small intestine (seven compartments), and there was no absorption within the stomach and colon compartments. Absorption within the seven mixing-tanks in series compartments for the small intestine was governed by the gastric emptying rate and different intestinal transit times (Yu et al., 1996; Yu and Amidon, 1998, 1999). Since the CAT model did not include the physical modeling of drug dissolution from dosage solid forms or controlled release formulations, degradation in lumen, changes in absorptive surface area, absorption in the stomach and colon, metabolism in liver, and transporter densities for absorption and efflux, a refined and expanded model, known as the advanced CAT or ACAT model was developed, with implementation of software, for the prediction of drug absorption (Agarwal et al., 2001). Details such as particle size, pH, particle density, and diffusion coefficient were included for consideration of drug dissolution and absorption. In another model, Ito et al. (1999) described intestinal metabolism and secretion, intracellular drug diffusion, and permeation through the basolateral membrane; however, the model seemed to lack description of intestinal flow. Moreover, these models do not relate to blood flow, drug partitioning, and the phenomenon of route-dependent intestinal metabolism. A greater understanding of the interplay will allow sound interpretations on intestinal drug metabolism and transport with respect to other drugs or the intake of fruit juices (Bailey et al., 1993; Lown et al., 1997a; Dremer et al., 2002).

Physiological Models: Traditional Model (TM) and Segregated Flow Model (SFM). Thus, the strategy is to turn to physiologically

based models that encompass all of the salient variables of transport, metabolism, efflux, gastrointestinal transit, and absorption (Doherty and Pang, 2000) (Fig. 2). The TM (Fig. 2A) was developed to describe data arising from recirculation of tracer morphine in the perfused rat small intestine preparation (Doherty and Pang, 2000). In this prepa-

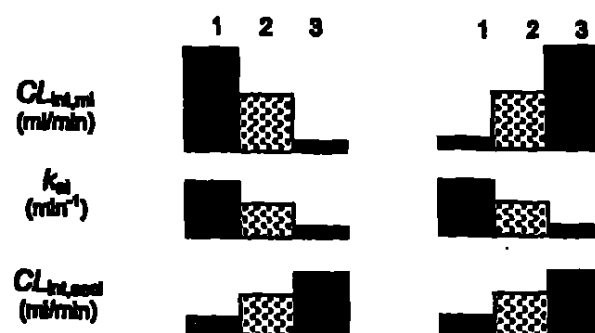


Fig. 6. Simulations based on intestinal modeling for the rat small intestine, to determine the varying distributions of absorptive and secretory transporters and metabolic enzymes that create high and low bioavailability.

Segmental distributions (designated as i^{th} segments where $i = 1, 2, \text{ and } 3$) of the metabolic intrinsic clearance, CL_{met} (0.9, 0.5, and 0.1 ml/min), the absorption rate constant, k_a (5, 3, and 1 min⁻¹), and the secretory intrinsic clearance, CL_{sec} (2, 4, and 6 ml/min) simulated the lowest bioavailability (F) (left panel) and the highest F (right panel; CL_{met} values were 2, 4, and 6 ml/min; CL_{sec} values were 0.1, 0.5, and 0.9 ml/min, and k_a values were 5, 3, and 1 min⁻¹) according to the predictions of the SFM and TM. The predicted differences existed in the descending versus ascending, segmental distribution of the metabolic intrinsic clearance, CL_{met} within the segments 1, 2, and 3. Taken from Tam et al. (2003b), with permission.

ration, morphine glucuronidation was observed with dosing of morphine into the duodenal lumen and not with administration into the reservoir that mimicked intravenous administration (Figs. 3 and 4). To address the apparent “inaccessibility” of the intestinal enzymes to the drug borne in the circulation but not lumen, or route-dependent metabolism, the segregated flow model, based on modifications of the model of Klippert and Norderhoek (1983) (Fig. 2B), was developed. SPM describes a partial and low blood flow to the “active” enterocyte region where the absorptive and excretory carriers and metabolic enzymes reside, and a much higher bulk flow to a nonabsorptive and nonmetabolizing region that included the serosa, submucosa, and mucosa, excluding the enterocyte region (Cong et al., 2000). Thus, the SPM recognizes the subtle denervation of these layers and distributions in blood supply (Svavrik, 1973; Granger et al., 1980). The literature values for the blood flow to the absorptive enterocyte layer

of the mucosa vary greatly, ranging from 5% to 30% (Miesfeldt et al., 1976; MacFarren and Mullman, 1977; Mullman 1978; Granger et al., 1980). Drug in the lumen must enter via the enterocyte region before reaching the circulation, whereas drug already in circulation is primarily channeled to other tissue regions. The condition conduces to a greater intestinal metabolism with oral versus intravenous dosing. For data on tracer morphine glucuronidation in the resecting, vascular perfused rat small intestine, the SPM was superior to describe the phenomenon of route-dependent intestinal metabolism (Cong et al., 2000). These physiological-based models developed for the intestine provide estimations of metabolism and MKT. Under linear conditions, intestinal secretion results in reduced intestinal metabolite formation, as confirmed in a recent theoretical examination of TM and SPM (unpublished data of K. S. Pang, R. Tseng, and D. Tam). The con-

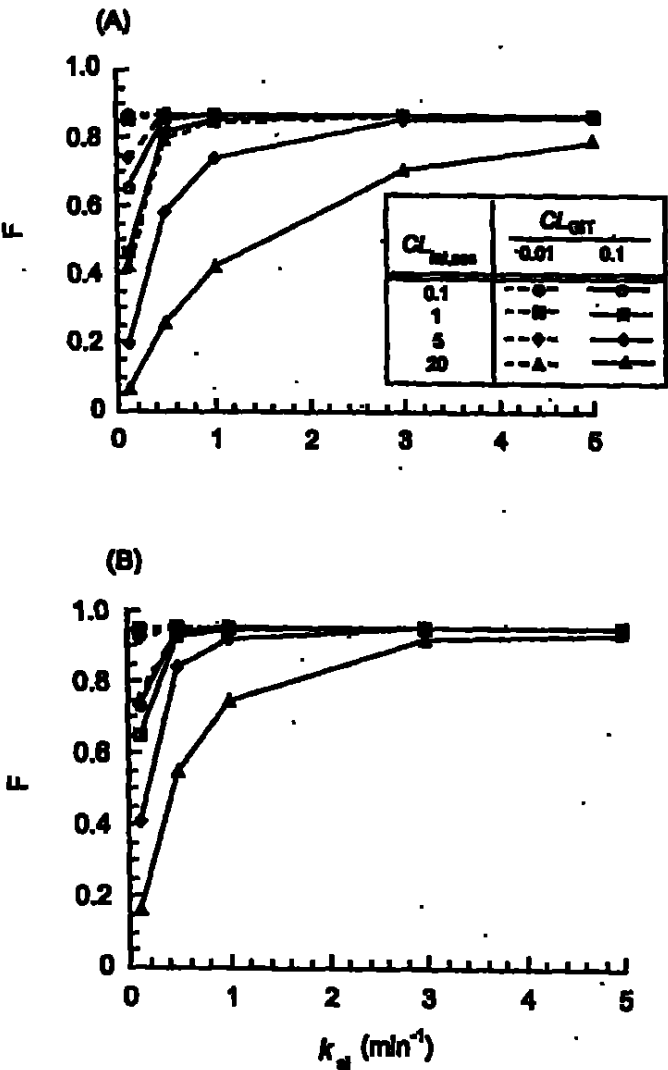


FIG. 7. Simulations performed for the STM (A) and SPM (B) to demonstrate the effect of the segmented, gastrointestinal tract clearance ($CL_{int} = 0.01$ or 0.1 ml/min), the secretory intrinsic clearance (CL_{secret}), and the absorption rate constant (k_a) on the intestinal (systemic) bioavailability, F . The metabolic intrinsic clearance, $CL_{int, seg}$ was kept at 0.1 ml/min; the partitioning clearance of drugs, CL_{int} and CL_{secret} , was 0.9 ml/min. All the intestinal segments ($i = 1, 2$, and 3) were assigned to be equal and contained equal amounts of transporter, metabolic, and absorptive activities. Adapted from Tam et al. (2003), with permission.

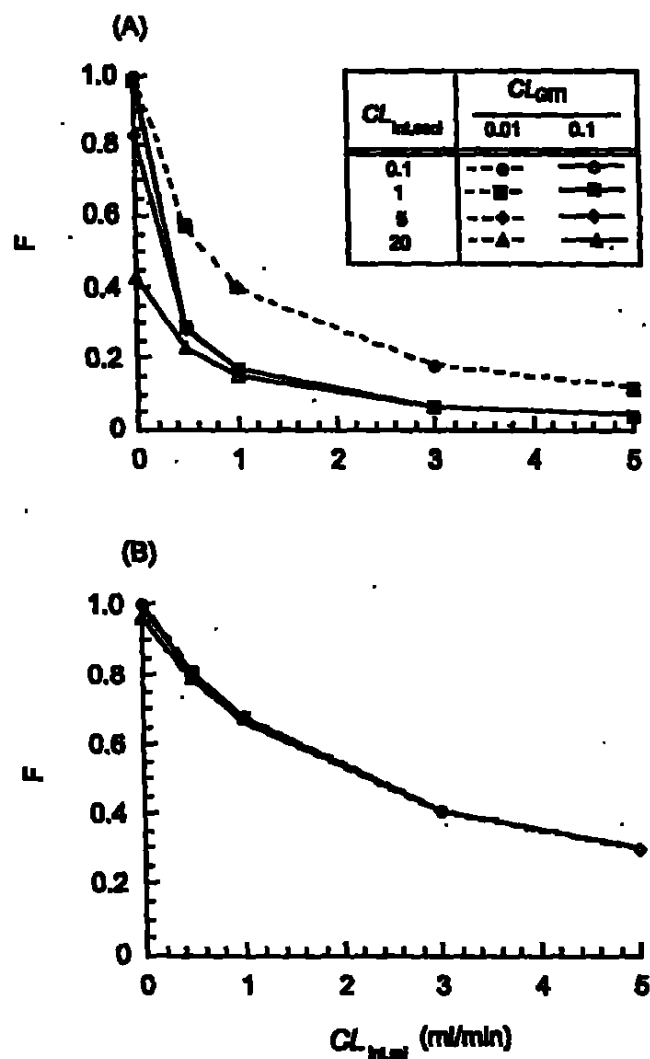


FIG. 5. Simulations performed for the STM (A) and SSFM (B) to demonstrate the effect of the gastrointestinal transit clearance ($CL_{trans} = 0.01$ or 0.1 ml/min), the secretory intrinsic clearance ($CL_{int,sec}$), and the metabolic intrinsic clearance (CL_{int}) on the intestinal (systemic) bioavailability, F . The absorption rate constant for the each segment, k_a , was kept at 3 min^{-1} ; the partitioning clearance for drugs, CL_{in} and CL_{out} , was 0.5 ml/min . All the intestinal segments (1 = 1, 2, and 3) were assigned to be equal and contained equal amounts of transporter, metabolic, and absorptive activities. Adapted from Tam et al. (2003), with permission.

clusion also agrees with intuitive and deductive reasoning since reduction of intracellular substrate concentration in the intestine accompanies drug efflux at the apical membrane, yielding lower rates of intestinal metabolism (Sirinoni and Pang, 1997; Schuster and Schinkel, 1999). This was also found in a simulation study based on Caco-2 cell efflux and metabolism (Tam et al., 2003a). Increased absorption neutralizes the effect of intestinal secretion and increased bioavailability (F), whereas increased secretion and metabolism reduces the bioavailability (Table 1).

Segmental, Traditional Model (STM) and Segmental Segregated Flow Model (SSFM). To accommodate the attendant heterogeneities of metabolic and transporter activities and to examine their impact on intestinal clearance and availability, more advanced models have been developed (Tam et al., 2003b). The single intestinal compartments of the TM and SPM were expanded into three equal

segmental compartments, as for the zonal modeling of the liver (Abu-Zehra and Pang, 2000), in the development of the segmental, traditional model (Fig. 5A) and the segmental, segregated flow model (Fig. 5B) (Tam et al., 2003b). The varying distributions of absorptive and secretory transporters and metabolic enzymes that evoked high and low availabilities were found from simulations based on intestinal modeling for the rat small intestine (Fig. 6). Of note is the strong influence of the distribution of the metabolic enzymes along the intestinal length on F. The predicted trends were generally similar for both STM and SSFM, although the magnitudes differed. When segments of equal activities for the transporters and metabolic enzymes were examined in a simulation study that viewed the intestine as the only eliminating tissue, a flat absorption, reflected by high absorption rate constants within the segments, counteracted the depth of the virtual, peripheral compartment and reduced the mean residence time

of drug in intestinal tissue, MRT_{int} . Rapid reabsorption of the secreted species canceled the effect of intestinal secretion and increased the F . However, high metabolic and secretion activities within the enterocytes greatly reduced F . A greater metabolic intrinsic clearance within the segments decreased MRT_{int} , whereas a greater secretory intrinsic clearance within the segments increased the MRT_{int} (Figs. 7 and 8) (Tan et al., 2003b).

Concluding Remarks and Future Modeling

Another question that remains is whether *in vitro* data would reflect intestinal metabolism *in vivo*. But data interpretation and correlation between *in vitro* and *in vivo* intestinal drug metabolism/removal is not straightforward. In addition to many of the usual problems often encountered for correlating *in vitro*-*in vivo* data on drug metabolism (Pang and Chiba, 1994), other variables are present, and these may further complicate the relationship. One reason is the efflux transporters at the apical membrane and the presence of intestinal motility that work in unison to remove drug out to the lumen, thereby preventing metabolism, although drug may be re-exposed to the enzymes upon reabsorption. Thus, not all of the effluxed drug is subject to intestinal metabolism, even though absorption is rapid. The second is route-dependent intestinal metabolism due to the peculiarity in intestinal blood flow to the "active" metabolic and absorptive region (Cong et al., 2000). The overall estimate of bioavailability is, therefore, complex, and is the consequence of membrane permeation by different arrays of transporters operating in the same or opposite directions, and cellular enzymatic activities that reduce the intracellular drug concentration.

The newly proposed physiologically based models that emphasize metabolism and secretion by the enterocytes have engendered many of the physiological processes governing the overall drug bioavailability and absorption. These models have provided tremendous insight into route-dependent intestinal metabolism/excretion, and the dynamic interplay among intestinal transit times, the absorptive carriers, and metabolic enzymes on intestinal clearance and bioavailability in the absence of a contribution by the liver. For application of the conceptual frameworks developed within the SPM and SSPM, the physiological parameters for the flow rate and volume, as well as more appropriate intestinal transit times, need to be properly scaled-up for modeling of human absorption.

The models, however, considered only drug already in solution. The complexities of drug dissolution and the physicochemical attributes of the various classes of drugs have yet to be integrated to the models. Mass balance with respect to ionization and absorption of the nonionic species may be further accommodated (Johnski et al., 1997). The dispersive surface area in drug absorption (Agarwal et al., 2001) and the intermittent release of drugs in various aggregated and deaggregated forms from the stomach, gastric emptying, bile salt effects, and the presence of mucus may need to be considered. Improvement in prediction of systemic availability will result upon coupling of the drug-release phase of the ACAT model (Agarwal et al., 2001). Sloughed off enterocytes that contribute to intestinal drug metabolism in lumen may need to be modeled (Gleason et al., 2002). Nonlinearity issues, in terms of intestinal drug-absorptive transporters, metabolic enzymes, and drug efflux transporters, must be considered in drug absorption, especially when the drug is absorbed rapidly to evoke saturation (Tamaai et al., 1997; Tan et al., 2003a).

The modeling of drug metabolism and transport by the intestine is at the early stages of development. Proof of the model relies not only on drug but also metabolite disposition during both oral and systemic dosing. Needless to say, the liver should be properly considered for first-pass removal and systemic bioavailability. It is expected that the

consolidation of physiologically sound intestinal and liver models will greatly improve our predictiveness on drug absorption bioavailability.

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Improvement of Dissolution and Oral Absorption of ER-34122, A Poorly Water-Soluble Dual 5-Lipoxygenase/Cyclooxygenase Inhibitor With Anti-Inflammatory Activity by Preparing Solid Dispersion

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ABSTRACT: Several formulation approaches were attempted to improve the dissolution and the oral absorption of ER-34122, which is a novel dual 5-lipoxygenase/cyclooxygenase inhibitor with potent anti-inflammatory activity. The solid dispersion of ER-34122 with hydroxypropylmethylcellulose (TC-5EW), which is an inert solid carrier, resulted in a significant improvement in the dissolution rate of ER-34122. The solid dispersion was prepared by a solvent evaporation method using ethanol and water. The solid-state characteristics of the solid dispersion, the corresponding physical mixture, and ER-34122 alone were investigated by X-ray powder diffraction, Fourier transform infrared spectroscopy (FTIR), and an automated controlled-atmosphere microbalance. The X-ray powder diffraction patterns suggest that the solid dispersion exists in a totally amorphous state and the others exist in a crystalline state. The FTIR spectra results suggest that ER-34122 can interact with TC-5EW through intermolecular hydrogen bonding in the solid dispersion. This interaction may cause a stabilization of ER-34122 in the higher-energy, faster-dissolving amorphous state. The dissolution rate of ER-34122 from the solid dispersion was significantly greater than that from the physical mixture or the pure drug. Furthermore, when orally administrated to beagle dogs, ER-34122 showed about a 100-fold increase in both maximum concentration (C_{max}) and area under the curve of concentration versus time (AUC) compared with the pure drug. Consequently, it was determined that the solid dispersion technique with TC-5EW provides a promising way to increase the dissolution rate and the oral absorption of poorly water-soluble drugs such as ER-34122. © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association *J Pharm Sci* 91:258–266, 2002

Keywords: ER-34122; solid dispersion; TC-5EW; dual 5-lipoxygenase/cyclooxygenase inhibitor; anti-inflammatory activity

INTRODUCTION

When a poorly water-soluble drug is orally administrated, its dissolution may be the rate-limiting step for its *in vivo* absorption and the

appearance of its medical effect. Therefore, a number of different formulation approaches^{1–3} have been investigated to increase the dissolution rate. One of the approaches is the formation of solid dispersions with pharmacologically inert, polymeric materials.^{4–7} Many solid dispersion techniques have been employed to enhance the dissolution rates of poorly water-soluble drugs, such as digoxin,⁸ ethenzamide,⁹ nifedipine,^{10–12} furosemide,^{12,14} griseofulvin,^{14,16} and tolbutamide.¹⁷

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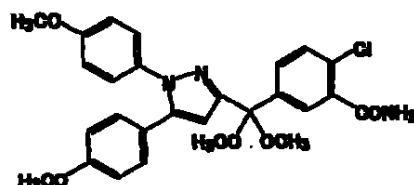


Figure 1. Molecular structure of ER-34122.

Furthermore, some promises and problems of such techniques for the manufacturing processes have been reported.¹⁸

ER-34122 (Fig. 1), a potent dual 5-lipoxygenase/cyclooxygenase inhibitor with anti-inflammatory activity,^{19,20} displays poor dissolution behavior in water because of its poor solubility in aqueous solution, as shown in Table 1. Based on an *in vivo* absorption study using an ER-34122 dose of 10 mg/kg in beagle dogs, the plasma level of ER-34122 following administration in a poly(ethylene glycol) 400 [(PEG) 400] solution, in which 10 mg/mL of this drug was soluble, was significantly higher than that of the pure drug. The area under the curve of concentration versus time (AUC) in the former and the latter were 4.943 ± 1.621 and 0.029 ± 0.012 $\mu\text{g} \cdot \text{h/mL}$. Accordingly, the dissolution was thought to be the rate-limiting step for the oral absorption of this drug.

In the present study, the dissolution enhancement of ER-34122 was attempted using a solid dispersion technique with TC-5RW, hydroxypropylmethylcellulose (Shinetsu Chemical Industry Company, Ltd., Tokyo, Japan) to improve the oral absorption. The prepared solid dispersion, the physical mixture, and ER-34122 alone were characterized by X-ray powder diffraction, Fourier transform infrared spectroscopy (FTIR), and a dissolution test. In addition, the extent of absorp-

tion for the solid dispersion and the pure drug was evaluated using beagle dogs.

EXPERIMENTAL SECTION

Materials

ER-34122, 5-[1,5-bis(4-methoxyphenyl)pyrazol-3-yl]dimethoxymethyl-2-chlorobenzamide, was synthesized at Tsukuba Research Laboratories for Drug Discovery, Eisai Company, Ltd. TC-5RW[®], hydroxypropylmethylcellulose, methylcellulose; methylcellulose (MC); and HP-55S[®], hydroxypropylmethylcellulose phthalate were purchased from Shinetsu Chemical Industry Company, Ltd. DE-88[®] and P-1570[®], which are sucrose esters of fatty acids, were purchased from Dai-ichi Kogyo Sanyaku Company, Ltd. (Kyoto, Japan) and Mitsubishi-kagaku Foods Corp. (Tokyo, Japan), respectively. PVP-VA64[®], poly(vinyl pyrrolidone) was purchased from BASF Japan, Ltd. (Tokyo, Japan). All other chemicals were of analytical grade, and the water used was filtered through a Mill-Q Water Purification System prior to use.

Methods

Solubility Studies

The aqueous solubility of ER-34122 was determined at 37°C. Excess solid ER-34122 was weighed and placed in a 5-mL screw-capped vial. After a 2-mL volume of the test medium was accurately added to each vial, the vial was tightly capped. The vials were then mechanically rotated in a constant temperature water bath (Yamato Scientific Company, Ltd., Tokyo, Japan) at 37°C for 24 h. After the sample solution was centrifuged at 3000 rpm for 10 min, the supernatant was filtered through a syringe filter (4 mm Millex-HV[®], 0.45 μm , Nihon Millipore, Ltd., Yonezawa, Japan). The filtrate was immediately diluted with a 50% (v/v) acetonitrile solution. The ER-34122 concentration in the filtrate, which was defined as the solubility in the test medium, was assayed by high-performance liquid chromatography (HPLC).

Preparation of Solid Dispersions and Physical Mixture

Six kinds of water-soluble polymers were used for the formulation studies of the solid dispersions. Each 1:5 (w/w) ER-34122/carrier dispersion was

Table 1. Solubility of ER-34122 in Various Media at 37°C

Tested Media		Solubility ($\mu\text{g/mL}$)
Species	pH	
Britton Robinson buffer	pH 2	≤ 0.01
Britton Robinson buffer	pH 3	≤ 0.01
Britton Robinson buffer	pH 4	≤ 0.01
Britton Robinson buffer	pH 5	≤ 0.01
Britton Robinson buffer	pH 6	≤ 0.01
Britton Robinson buffer	pH 8	≤ 0.01
JP 1 medium	pH 1.2	≤ 0.01
JP 2 medium	pH 6.8	0.03

prepared by the solvent evaporation technique, wherein 5 g of the appropriate ratio of the two components was dissolved in 200 mL of 80% (w/w) ethanol/water at 50°C. As for the 1:0.5, 1:1, and 1:2 (w/w) ER-34122/TC-5RW dispersions, 3 g of the appropriate ratio of the two components was dissolved in 300 mL of 87% (w/w) ethanol/water at 50°C. The solvents were removed under reduced pressure using a rotary evaporator at 50°C. After any residual solvents were removed by drying under vacuum at room temperature, the solid dispersions were ground into powders with a mortar and pestle. The 1:1 (w/w) physical mixture of ER-34122 and TC-5RW was prepared by thorough mixing in an agate mortar with a pestle. All the powdered samples were used for the following studies after passing through a sieve (sieve size: 150 μ m).

X-ray Powder Diffraction (XRD)

XRD patterns of the ER-34122 test preparations were measured with a Rigaku RINT-2500 Ultrax18 X-ray diffractometer (Rigaku, Tokyo, Japan). The radiation was generated by CuK α with a wavelength of 1.5418 Å at 40 kV and 200 mA. Each sample was packed into a copper holder, and the instrument was operated in the continuous scan mode over the 2 θ range of 5 to 30°.

Dissolution Studies

A JP XIII Type 2 apparatus (rotating paddle method) was employed to obtain all the dissolution profiles of ER-34122 alone, the physical mixture, and the solid dispersions. Each powdered sample contained 10–20 mg of ER-34122. The dissolution test was carried out at 37 $\pm$ 0.5°C at a rotation speed of 50 rpm. The dissolution medium was 900 mL of JP XIII disintegration second medium (JP 2 medium, pH 6.8). Aliquots were withdrawn at appropriate time intervals and immediately replaced with an equal volume of fresh dissolution medium. The concentration of ER-34122 in the medium was determined by HPLC.

HPLC Analysis

The HPLC analyses were performed with a Shimadzu LC10A system (Shimadzu, Kyoto, Japan) equipped with a 4.6 $\times$ 250 mm YMC-Pack ODS AM-308 column which was maintained in a column oven at 35°C. The mobile phase consisted of a 50% (v/v) mixture of acetonitrile and water.

The flow rate was 1.0 mL/min, and the detection wavelength was 254 nm.

FTIR Analysis

FTIR spectra were recorded with a Jasco FT/IR-630 spectrometer (Jasco, Tokyo, Japan). Samples were scanned as potassium bromide pellets with an instrument resolution of 4 cm⁻¹. Data were collected over the spectral region from 4000 to 400 cm⁻¹.

Hygroscopicity Measurements

Vapor pressure isotherm data for ER-34122 alone and the 1:1 (w/w) drug/TC-5RW dispersion were obtained using an automated controlled-atmosphere microbalance (VTI Corporation Hialeah, FL). In this system, the sample was suspended in an isothermal chamber at 25°C, and the relative humidity (RH) was regulated from 5 to 95% by adjusting the relative flow rates of dry (0% RH) and moist (100% RH) nitrogen. The weight of the sample was measured every 2 min with the microbalance. The weight stability criterion employed for the equilibrium was that the maximum weight change for each measurement was <0.2% (w/w).

In Vivo Absorption Studies

The *in vivo* absorption studies of ER-34122 alone and the 1:1 (w/w) drug/TC-5RW dispersion were carried out using male beagle dogs ($n = 3$). Each dog was fasted overnight, then a capsule (No. 000) containing the pure drug or the solid dispersion (equivalent to 10 mg/kg dose of ER-34122) was orally administered with 50 mL of water. After the dose administration, the dogs were kept in restraining cages, and access to food and water was allowed after 8 h. Serial blood samples (2-mL aliquots) were taken from the dogs with heparinized syringes at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post administration. The blood samples were immediately centrifuged, and the plasma samples were stored at -20°C until assayed. The concentration of ER-34122 in the plasma was assayed by HPLC, following the procedure illustrated in Chart 1. The calibration curves of ER-34122 covered concentrations over the 0–1 μ g/mL range. The AUC was determined as the area under the plasma concentration–time curve up to the last measured time point calculated by the trapezoidal rule, plus the residual area calculated as the concentration of the last measured time point divided by the elimination rate constant.

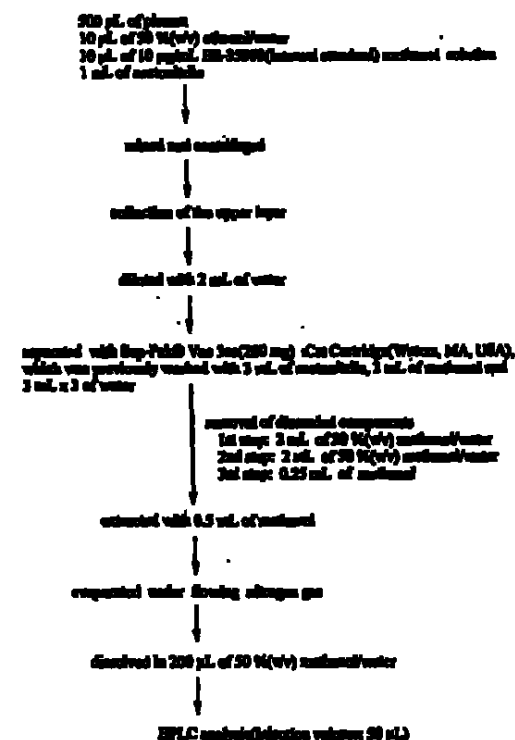


Chart 1. Pretreatment flow of plasma sample for HPLC analysis of ER-34123.

RESULTS AND DISCUSSION

Preparation of Solid Dispersion

For the formulation studies of the solid dispersions, 1:5 (w/w) drug/carrier dispersions were prepared using six kinds of water-soluble polymers. The dissolution rates of ER-34123 from these solid dispersions, which showed no diffraction peaks in the XRD patterns, were significantly faster than that from the pure drug,^{14,21-23} however their dissolution profiles were different (Fig. 2). TC-5RW was chosen as the best carrier for further studies because it resulted in the highest increase in drug dissolution.

The effects of the TC-5RW content on the release of ER-34123 from the solid dispersions are shown in Figure 3. Four levels of drug-to-carrier ratios [i.e., 1:0.5, 1:1, 1:2, and 1:5 (w/w)] were studied. The increase in the polymer content enhanced the dissolution of the drug but it caused a large dosage form. Considering the balance of the dissolution profile and the dosage form, the 1:1 (w/w) drug/TC-5RW dispersion was thought to

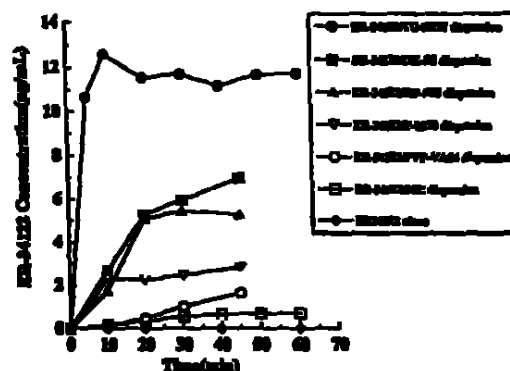


Figure 2. Dissolution profiles of ER-34123 from 1:5 (w/w) drug/carrier dispersions in JP 2 medium at 37°C. Six kinds of water-soluble polymers (i.e., TC-5RW, DE-SE, HP-568, P-1570, PVP-VA64, and MC) were used for the preparation of 1:5 (w/w) drug/carrier dispersions.

be the most suitable for the ER-34123 dosage form.

Physicochemical Property

The XRD patterns for ER-34123 alone, the 1:1 (w/w) drug/TC-5RW dispersion, and the corresponding physical mixture are shown in Figure 4. ER-34123 is crystalline, as demonstrated by its sharp and intense diffraction peaks, whereas the solid dispersion showed no diffraction peaks, suggesting that ER-34123 was in the amorphous state.⁷ In contrast, the physical mixture showed several diffraction peaks attributable to the crystalline form of the drug.

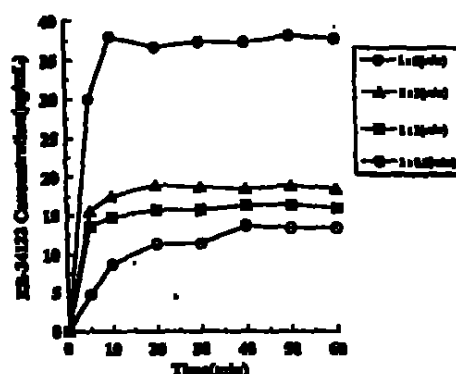


Figure 3. Effect of TC-5RW content on the release of ER-34123 from solid dispersions. Drug/TC-5RW dispersions with four levels of TC-5RW content [1:0.5, 1:1, 1:2, and 1:5 (w/w)] were studied.

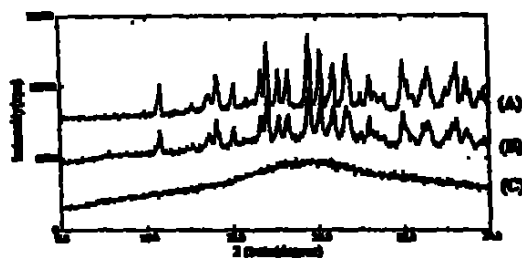


Figure 4. XRD patterns of ER-34122 alone, 1:1 (w/w) ER-34122/TC-5RW dispersion, and the corresponding physical mixture: (A) ER-34122 alone, (B) the physical mixture, and (C) the solid dispersion.

The FTIR spectra of ER-34122 alone, the 1:1 (w/w) drug/TC-5RW dispersion, and the corresponding physical mixture are shown in Figure 5. Below 2000 cm^{-1} , no difference was observed between the drug and the physical mixture, whereas the solid dispersion showed a different spectrum. Such differences have been observed in other solid dispersion techniques.^{24–26} From the FTIR spectra of ER-34122, an absorption band was observed at 1667 cm^{-1} , which is assigned to the C=O bond stretching vibration of Ar-CONH₂.²⁷ The absorption band was upshifted to 1684 cm^{-1} in the solid dispersion. In the physical mixture, however, the corresponding absorption band did not shift. This result implies that intermolecular hydrogen bonding occurs between

the N-H and C=O moieties of ER-34122 in its undiluted form, whereas in the solid dispersion, the binding is spread out. The C=O functional groups of ER-34122 may interact with the functional groups (e.g., hydroxyl) of TC-5RW at the molecular level in the solid dispersion like other solid dispersion systems,^{10,11} which use hydroxypropylmethylcellulose, that also improved the dissolution profiles of nifedipine, which contains such moieties. This interaction may also cause a stabilization of ER-34122 in the higher-energy, amorphous state.²⁸

The difference in hygroscopicity between ER-34122 alone and the 1:1 (w/w) drug/TC-5RW dispersion are shown in Figure 6. ER-34122 was hardly hygroscopic, whereas the solid dispersion adsorbed 2% (w/w) moisture when equilibrated at 75% RH. Because the hygroscopicity was reversible and no crystallization was observed by XRD (data not shown), the solid dispersion proved physically stable under the humid conditions.

Solubility and Dissolution Profile

The dissolution profiles of ER-34122 alone, the 1:1 (w/w) drug/TC-5RW dispersion, and the corresponding physical mixture in JP 2 medium at 37°C are shown in Figure 7. The solid dispersion exhibited faster dissolution rates than ER-34122 alone or the physical mixture. Because the solubility of ER-34122 in the medium at 37°C

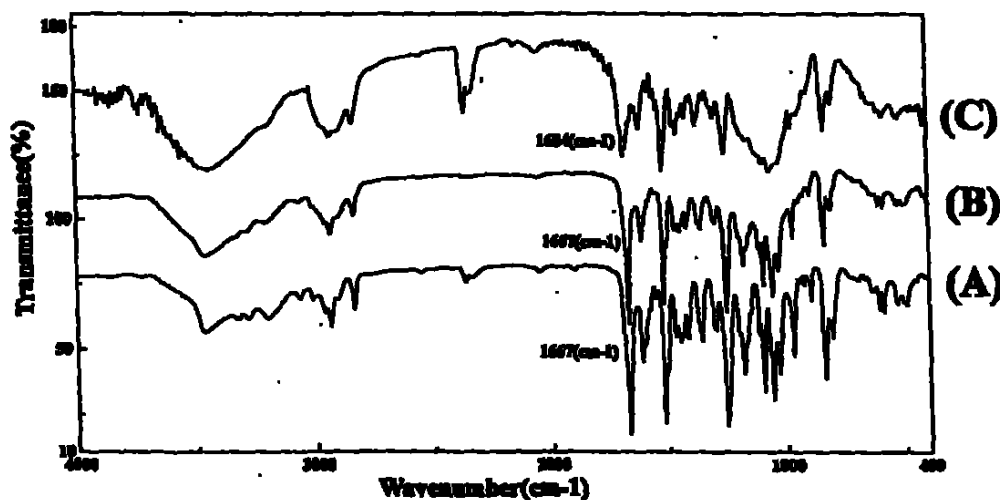


Figure 5. FTIR spectra of ER-34122 alone, 1:1 (w/w) ER-34122/TC-5RW dispersion, and the corresponding physical mixture from 4000 to 400 cm^{-1} : (A) ER-34122 alone, (B) the physical mixture, and (C) the solid dispersion.

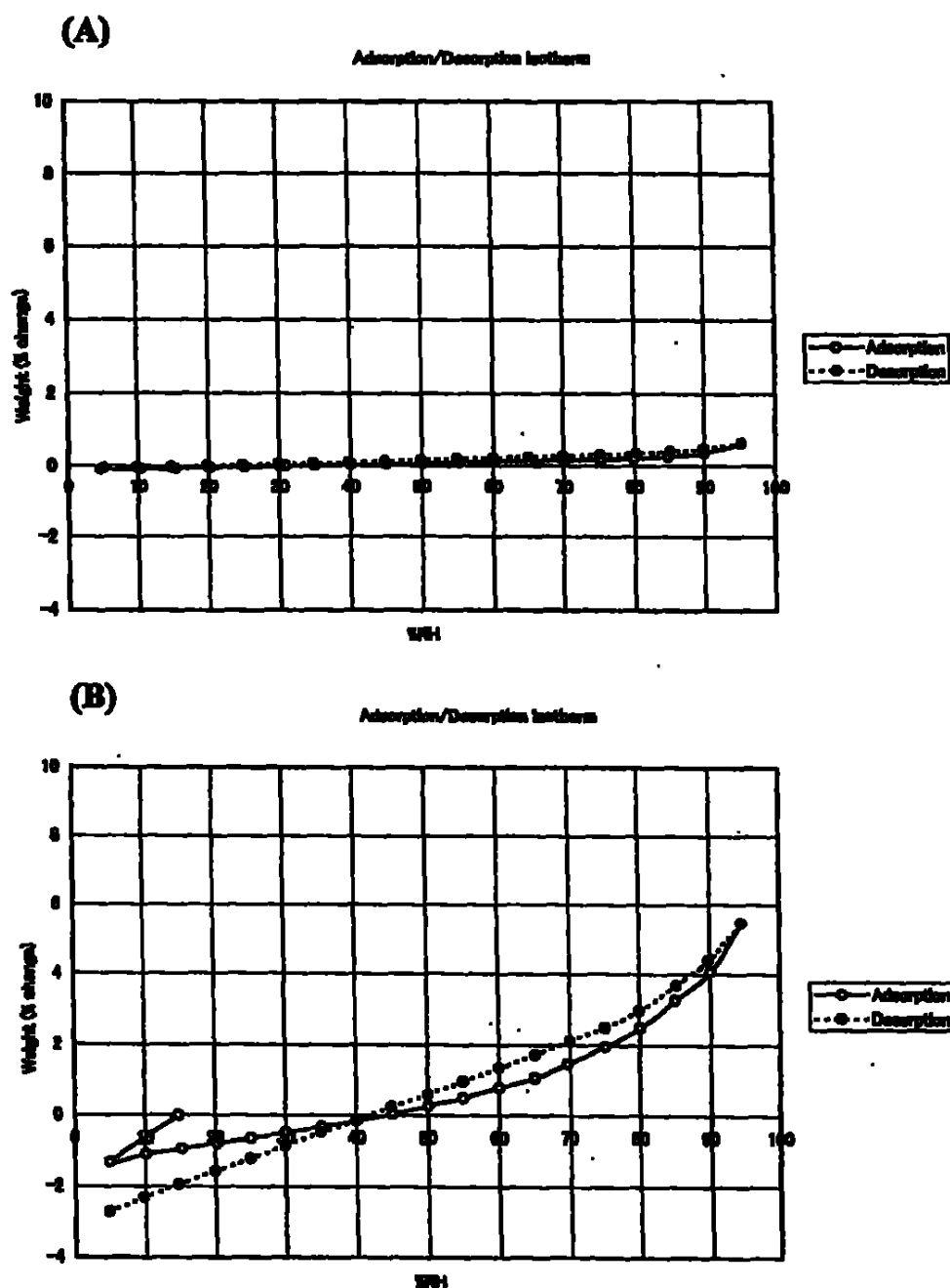


Figure 8. Adsorption-desorption isotherm for ER-34122 alone and 1:1 (w/w) ER-34122/TC-5RW dispersion: (A) ER-34122 alone and (B) the solid dispersion.

was 0.03 $\mu\text{g/mL}$ (Table 1), the concentration following the dissolution of the solid dispersion and the physical mixture indicated supersaturation. The supersaturated solution was stable, and

recrystallization did not occur under the experimental conditions. The reason why dissolution of the physical mixture was superior to that of the drug alone may be that TC-5RW inhibits the

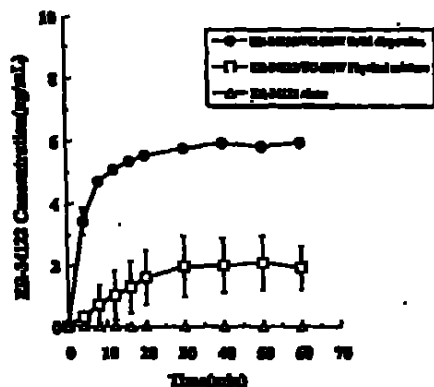


Figure 7. Dissolution profiles of ER-34122 alone, 1:1 (w/w) ER-34122/TC-5RW dispersion, and the corresponding physical mixture in JP 2 medium at 37°C. The test was carried out in triplicate.

precipitation of ER-34122 or lowers the surface tension of the medium,²⁸ resulting in better wettability.^{2,7,20} On the other hand, the remarkable increase in drug release from the solid dispersion demonstrates the interaction between the drug and the carrier.

In Vivo Absorption

ER-34122 in the 1:1 (w/w) drug/TC-5RW dispersion was expected to exhibit a better bioavailability because of its greater dissolution rate.^{21–23} The mean plasma levels and the pharmacokinetic parameters of ER-34122 when the drug alone and the solid dispersion were orally administrated to beagle dogs are shown in Figure 8 and Table 2, respectively. The plasma level of ER-34122 following the administration of the solid dispersion was significantly higher than that of ER-34122 alone up to 24 h. Both the C_{max} and AUC of the solid dispersion were ~100 times higher than those of the pure drug, indicating a remarkable improvement in the oral absorption. Accordingly, ER-34122 was thought to rapidly dissolve in the gastrointestinal fluid and be well absorbed when it was administrated as the solid dispersion. Though the ER-34122 concentration in the fluid may exceed its solubility, it would be effectively absorbed without recrystallization. In the case of ER-34122 alone, the dissolution was thought to be the rate-determining step in the absorption process, because it could not be orally absorbed very well.

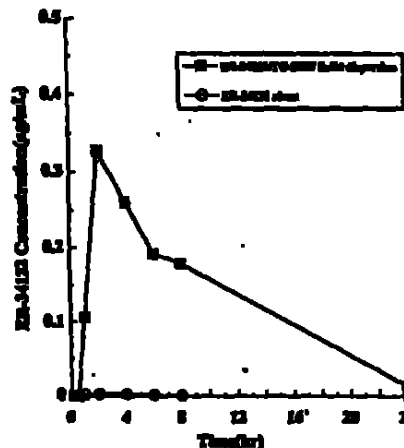


Figure 8. Plasma levels of ER-34122 after the pure drug and 1:1 (w/w) drug/TC-5RW dispersion were orally administrated at the dose of 10 mg/kg as ER-34122 to beagle dogs ($n = 3$).

CONCLUSION

The dissolution of ER-34122 from a 1:1 (w/w) drug/TC-5RW dispersion was significantly increased, as shown in Figure 7. In the XRD pattern of the solid dispersion, ER-34122 was detected in the amorphous state, which proved to be physically stable. ER-34122 was also thought to interact with TC-5RW through intermolecular hydrogen bonding in the solid dispersion. Both the C_{max} and AUC of the solid dispersion were ~100-fold higher than ER-34122 alone in beagle dogs. Therefore, it can be concluded that the solid dispersion technique using TC-5RW, which is a conventional excipient, would be a useful method to enhance the oral absorption of ER-34122, whose solubility is much less than other poorly water-soluble drugs introduced on the market.^{24,25} Also, this technique can be valuable for

Table 2. Pharmacokinetic Parameters of ER-34122 After the Pure Drug and 1:1 (w/w) Drug/TC-5RW Dispersion Were Orally Administrated at the Dose of 10 mg/kg as ER-34122 to Beagle Dogs ($n = 3$)^a

Parameter	ER-34122 Alone	ER-34122/TC-5RW Solid Dispersion
AUC ($\mu\text{g} \cdot \text{h/mL}$)	0.029 ± 0.012	3.291 ± 1.161
MRT (h)	4.98 ± 0.59	6.59 ± 1.04
C_{max} ($\mu\text{g/mL}$)	0.004 ± 0.001	0.328 ± 0.100
T_{max} (h)	2.00 ± 0.00	2.00 ± 0.00

^aEach value represents the mean $\pm$ SEM.

drug discovery and development where the solubility of compounds is one of the key factors.

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The relative bioavailability and pharmacokinetics of carbamazepine¹

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Differences in the bioavailability after administration of two pure carbamazepine samples that were different in particle sizes have been found. Carbamazepine was given to each of 3 healthy male volunteers in a single oral dose of 400 mg in gelatin capsules. The other carbamazepine sample was administered after a three-week time interval. Plasma was collected during the following 96 hr. Quantitative determination of carbamazepine was performed by gas liquid chromatography. By comparing the areas under the various curves for each volunteer, the relative bioavailability of one of the carbamazepines in relation to the other one was found of approximately 70%. There were no differences in the half-life time.

The bioavailability of drugs is dependent on factors which influence the resorption of the drugs. If there is a low aqueous solubility, for example, in carbamazepine, the velocity of resorption might be limited by the dissolution rate, which, in turn, would depend on the particle sizes of the drug [3, 5, 6]. In connection with other problems we compared the bioavailability of two pure carbamazepine samples which were different in the distribution of particle sizes.

Material and methods

Each of 3 healthy male volunteers who were 34 years old and weighed from 74 to 84 kg was given orally in a crossover study 400 mg carbamazepine in gelatin capsules. The second carbamazepine were administered after a four-week time interval.

The drug was administered to the fasting volunteers in the morning two hr before a light breakfast. Blood samples were collected from the antecubital vein just before and 1, 2, 3, 4, 6, 8, 10, 12, 24, 28, 32, 48, 52, 56, 72, 76, 80 and 96 hr after the drug intake.

The 2 carbamazepine samples were distinguished by the numbers 1 and 2 respectively.

Carbamazepine 1: VEB Arzneimittelwerk Dresden, D.D.R. (Finlepain®)

Carbamazepine 2: unknown producer

Analytical procedure

One ml of plasma was diluted with 0.5 ml of water and then extracted twice with 4 ml of diethylether. The ether phases were evaporated, and the residue was redissolved in 3 ml of a mixture of hexane/ethanol (10:4). After the

addition of distilled water (200 µl) and shaking for 10 min, the hexane phase was discarded and the ethanol-water phase evaporated to dryness. The residue was redissolved in a suitable volume of acetone for gas chromatographic determination. Diazepam was added to the solvent as a volume standard. Plasma containing known amounts of carbamazepine was used for the determination of the calibration curves for each set of samples.

The analysis was carried out on a Varian gas chromatograph 2740 equipped with a flame ionization detector. Column packing was 5% QV 17 on Varaport 30 in a 3-ft glass column of 2 mm inner diameter. The carrier gas was nitrogen with a flow rate of 25 ml/min. The temperatures were column, 220°C; injector, 250°C; and detector, 300°C.

Thermal disintegration of carbamazepine to iminostilbene was not observed under these conditions.

All the samples were made in duplicates. The standard deviation was 10%.

The calculation of the areas under the concentration time curves (AUC) was performed by the trapezoidal rule on a table calculator (Hewlett-Packard, type 9820 A, program Model 20, Math. pac. III-5).

Results

The carbamazepine plasma levels of the three subjects A, B and C are shown in Figure 1. There are interindividual differences in the half-life times, but there are no statistically significant differences ($p < 0.05$) for each single subject (paired t-test [1]).

The half-life times (Table 1) calculated from the terminal slope (24-96 hr) agree with the data reported in the literature [2, 4, 6].

¹ Paper presented at the 2nd Symposium on Clinical Pharmacology, Poznań, Poland, May 6-7, 1977.

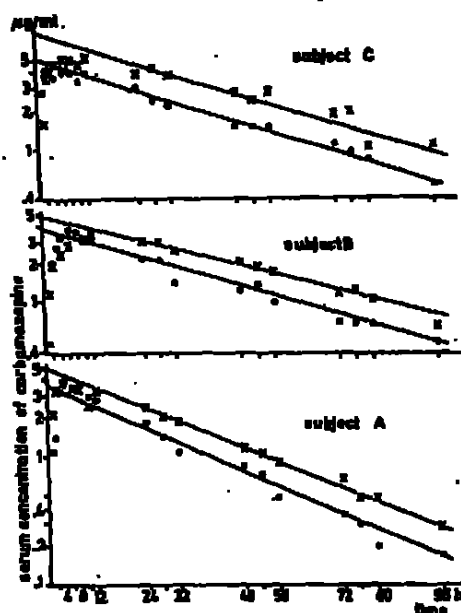


Figure 1. Plasma concentrations of carbamazepine in 3 healthy volunteers following oral administration of 400 mg each of carbamazepine 1 and 2.

Subject	Half-life times [hr]	
	carbam. 1	carbam. 2
A	20.8	24.3
B	31.1	36.8
C	29.5	32.5

Table 1. Calculated half-life times of carbamazepine 1 and 2 in 3 volunteers following oral administration of 400 mg.

Subject	AUC ($\mu\text{g} \times \text{hr} \times \text{ml}^{-1}$)		Rel. bioavailability of carbamazepine 1
	carbam. 1	carbam. 2	
A	105	154	68%
B	143	198	72%
C	187	276	68%

Table 2. The relative bioavailability and the area under the plasma AUC in 3 volunteers following the oral administration of 400 mg each of carbamazepine 1 and carbamazepine 2.

The area under the AUC was taken as representative of the bioavailable amount of the drug. The calculated AUCs are presented in Table 2. The relative bioavailability of carbamazepine 1 was found to be approximately 70% of carbamazepine 2 in all 3 volunteers ($p < 0.05$).

The mean values of the carbamazepine plasma levels are depicted in linear scales in Figure 2. As one can see, the greater AUC of carbamazepine 2 is caused only by a



Figure 2. Mean values of the plasma carbamazepine levels of sample 1 and sample 2 in 3 volunteers following oral administration of 400 mg.

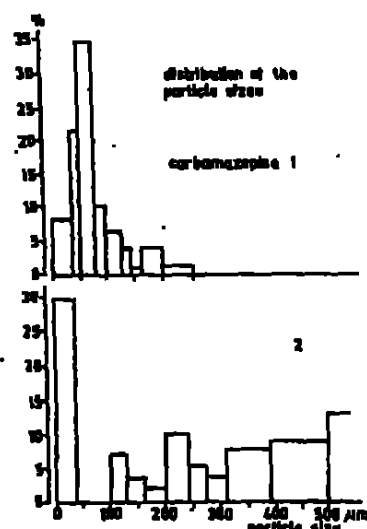


Figure 3. Distribution of particle sizes of carbamazepine 1 and carbamazepine 2.

longer resorption time. The peak concentrations are nearly the same.

The distribution of the particle sizes of the carbamazepine samples was determined by a set of sieves. The results are shown in Figure 3.

Discussion

It has become evident in recent years that the gastrointestinal absorption of drugs is dependent on various factors. The dissolution rate is an important factor, especially for drugs of a low aqueous solubility, which influences the bioavailability [5]. However, the short disintegration time of tablets or capsules will be of a lower effect.

We found a lower bioavailability of carbamazepine 1 in each of the 3 subjects. This result could not be caused by

induction of the carbamazepine metabolism because the experiments were carried out in a crossover study and the half-life times were not changed.

But there was a different distribution of particle sizes. Carbamazepine 1 has only a relatively small variation in particle sizes, whereas carbamazepine 2 consists of particles with very different diameters. From this point of view, one might expect that carbamazepine 1 will be dissolved and absorbed rather quickly and more completely than carbamazepine 2. But in practice we found a

longer resorption time for carbamazepine 2 and therefore a higher bioavailability.

From these facts we must conclude that there are still other unknown properties of carbamazepine crystals which influence the bioavailability.

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Relationship Among Particle Size Distribution, Dissolution Profile, Plasma Values, and Anthelmintic Efficacy of Oxfendazole

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SUMMARY

Three mean particle sizes of oxfendazole raw material (1.65 μm , lot A; 3.2 μm , lot B; 12.0 μm , lot C) were prepared and identically formulated as corresponding (A, B, and C) suspensions at 2.26% (w/v) concentration. Studies involving microscopic examination, scanning electron microscope analysis, particle size distribution, and surface area measurement were carried out on raw materials. In vitro dissolution profiles were obtained for the suspensions. A comparative bioavailability study of these 3 suspensions was performed in 12 sheep with each sheep given each formulation in a Latin square crossover study design; oxfendazole was dosed at a rate of 5 mg/kg of body weight. Plasma-value measurements were made followed by an analysis of various bioavailability studies. Plasma area values indicated that suspension C (dw = 12.0 μm) was significantly ($P < 0.05$) less bioavailable than was suspension A (dw = 1.65 μm); there was no difference between suspension A and suspension B. Significant differences were not seen in biological half-life and maximum plasma concentrations.

The term dw refers to that particle diameter (determined by Coulter counting) at which 50% of the oxfendazole mass was in the form of parti-

cles having a lesser diameter and 50% was in the form of particles having a greater diameter.

In a separate study involving 20 Marino weaner sheep infected with benzimidazole-resistant *Haemonchus contortus* larvae, oxfendazole's anthelmintic efficacy was demonstrated in the 2.26% suspension dosage form (90% particles < 10 μm) at a dosing rate of 5 mg/kg. A correlation was found between its anthelmintic activity and plasma area values when compared in individual sheep.

Data demonstrated that substantial differences in particle size distribution of oxfendazole could influence its dissolution rate, plasma concentrations, and absorption characteristics, thus indicating that oxfendazole's absorption could be dissolution-rate limited.

Oxfendazole, methyl 5-(phenylsulfinyl)-1*H*-benzimidazol-2-yl carbamate, is a systemic anthelmintic intended for oral use in a variety of animal species, including horse, sheep, and cattle. It has been clinically effective against a broad range of nematodes, including strains of *Haemonchus contortus*, previously known to be resistant to many other benzimidazole anthelmintics.¹⁻³ The drug has a relatively low aqueous solubility of less than 4 $\mu\text{g}/\text{ml}$ at a pH of 7.8. It is known that the particle size of poorly soluble drugs, such as griseofulvin, can

be of critical importance in gastrointestinal absorption and subsequent bioavailability and pharmacologic effect in persons. This appeared to be a dissolution rate limited phenomenon. Particle size reduction in such cases resulted in an increase in specific surface area and a corresponding higher dissolution rate.^{4,5} Particle size distribution is a major factor in the efficiency of phenothiazine and some other benzimidazole derivatives as anthelmintics in ruminants.⁴⁻⁶

The purpose of the present study was to investigate the interrelationship among particle size, surface area, dissolution rate, plasma values, and anthelmintic efficacy of oxfendazole administered as a suspension to sheep.

Materials and Methods

Three lots of the drug substance with different particle size distributions and surface areas were prepared by using appropriate techniques and were incorporated into suspension dosage form. Oxfendazole dissolution rates were examined in the finished suspensions. The bioequivalence of these suspensions was determined in a 12-subject crossover study in normal sheep. In a separate study, the suspension dosage form was administered to 12-month-old Marino weaner sheep reared worm-free and subsequently infected with *H. contortus* larvae. Plasma oxfendazole concentrations were monitored, and the efficacy of the oxfendazole in individual sheep was determined by comparison of worm counts with the geometric mean count in the control sheep.

Preparation of Drug Substances of Different Average Diameter—Three lots of oxfendazole drug substance with particle size of different weight mean diameter (dw; in microns) were manufactured as follows: lot A of oxfendazole was prepared by milling a batch of oxfendazole drug

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From Syntex Research, Palo Alto, CA 94304 (Shastri, Mrozczek, Parekh, Nguyen, Schiltz), and Commonwealth Scientific and Industrial Organisation, Division of Animal Health, McMaster Laboratories, Sydney, Australia (Prichard, Hennessey).
The authors thank Mr. J. Allen and Dr. D. Black for technical assistance.

* Demonstration of efficacy against *Beshtopsis* of this benzimidazole-resistant *H. contortus* (unpublished data, Syntex Research, Palo Alto, Calif.).

substances³ through a micropulverizer⁴ and by subsequently passing it through a microsizer.⁵ The milled material was termed lot B before micronization. Lot C was prepared by using the following technique: 600 g of oxendazole raw material was mixed with 10,000 ml of deionized water containing 5 g of polyvinylpyrrolidone-40-monoacetate.⁶ The suspension was passed through a 20- μ m nylon fabric screen.

The material passing through the 20- μ m screen was then passed through a 10- μ m screen. Oxendazole left behind on the screen was washed with water, was filtered over a sintered glass funnel, and was dried in an oven overnight at 40 C. Soft, powdery lumps obtained by this process were passed through a 35-mesh screen to convert them into a uniform powder. (It was termed coarse in a relative sense to differentials it as being less finely divided than were lots A and B. In reality, lot C also contained fine particles.)

These particle size reduction techniques were used to obtain 3 lots of potentially different particle size distributions and to study the influence of micronization on raw material characteristics. Suspensions containing 2.26% oxendazole were prepared identically with these raw materials and were labeled as A, B, and C, corresponding respectively to lots A, B, and C.

Particle Size Distribution Analysis of Raw Material Lots—Preliminary sizing experiments on the raw material lots were made by examination under a microscope⁷ attached with a calibrated ocular micrometer and phase-contrast equipment. These studies revealed that in all the tested lots, only occasionally was a particle larger than 30 μ m in diameter. This information was helpful in selecting the appropriate aperture tubes for Coulter counter runs.

Scanning electron microscope (SEM) analysis⁸ of the 3 lots was performed as follows: Clean adhesive tape was placed on a flat piece of aluminum which in turn was fitted onto a clean mounting stub. A sample from each lot of oxendazole powder was then sprinkled onto the tape and was coated with gold, using a sputter coater. The gold film was approximately 10 nm thick. The sample was then observed under the microscope⁹ and was photomicrographed at 2,000 $\times$ and 5,000 $\times$ magnification. The SEM was calibrated every month with a size accuracy of 5%.

Particle size distribution was examined with a Coulter counter¹ equipped

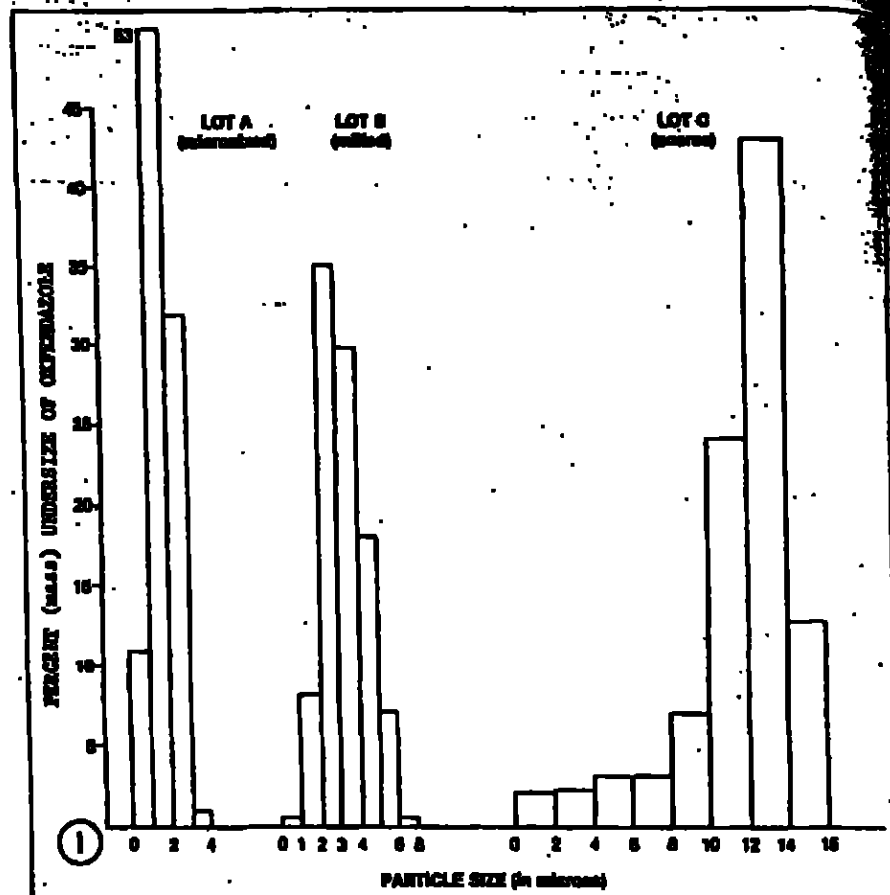


Fig 1—Particle size distribution of oxendazole lots by Coulter counter measurements.

with a triple nanometer and a 60- μ m, as well as a 100- μ m aperture tube. The instrument was previously calibrated with 2.02- μ m polystyrene latex microspheres supplied by the manufacturer. A sample (approx 5 mg) was transferred to a 30-ml plastic cuvette and was wetted with a few drops of 10% polyvinylpyrrolidone-40-monoacetate solution in water that had been previously filtered through a 0.4- μ m membrane filter.¹⁰ It was adjusted to volume (20 ml) with 0.9% NaCl-azide free balanced electrolyte isotonic solution,¹¹ and was suspended uniformly by shaking for 15 s in a sonic bath. A small portion of this suspension was transferred to another 30-ml cuvette and was adjusted to 20 ml with the aforementioned isotonic solution. This suspension was ready for particle count. Approximately 50,000 particles were counted per analysis. Tube clogging was not experienced during the analyses.

The percentage of weight distribution for each size interval was read directly from the Coulter counter. The differential weight percentage and cumulative weight

percentage histograms were obtained, using an x-y recorder. A frequency distribution respective vs particle size is represented by a histogram shown in Figure 1, whereas a linear cumulative plot on probit scale is outlined in Figure 2. The d_w (that particle diameter, determined by Coulter counting, at which 50% of the oxendazole mass was in the form of particles having a lesser diameter and 50% was in the form of particles having a greater diameter) was determined by reading off 50% values of the distribution. All lots were evaluated in this manner. Five individual sample runs were carried out for each raw material lot.

Surface-Area Measurements—Oxendazole powders were dried for 24 hours in a vacuum oven and were subsequently flushed with nitrogen for 30 minutes. The surface areas (m^2/g) were obtained by single-point Brunauer, Emmett, and Teller (BET) determination on an isothermal adsorption surface area analyzer, using nitrogen-helium mixture as adsorbate.¹² Three independent runs were carried out on each lot of oxendazole. This measurement of specific surface area was based on an adsorption isotherm equation derived by BET. Essentially, the effective surface

¹ Syntex Corp, Palo Alto, Calif.

² Milliron, WG Martens & Co, Chatham, NJ.

³ Millman Master, Jet Pulverizer Co, Pulayon, NJ.

⁴ ICI America Inc, Wilmington, Del.

⁵ TETCO Inc, Elmsford, NY.

⁶ Ernst Leitz Inc, Wetzlar, Germany.

⁷ Measurements performed by Surface Science Laboratories Inc, Palo Alto, Calif.

⁸ Cambridge model 600, Cambridge Instruments, Cambridge, England.

⁹ Model TAI, Coulter Electronics Inc, Elkhart, Fla.

¹⁰ Polycarbonate 0.45- μ m filter membranes, Nalco Corp, Naperville, Ill.

¹¹ Isoton II, Coulter Diagnostics, Division of Coulter Electronics, Elkhart, Fla.

¹² Quantachrome Corp, Germantown, NY.

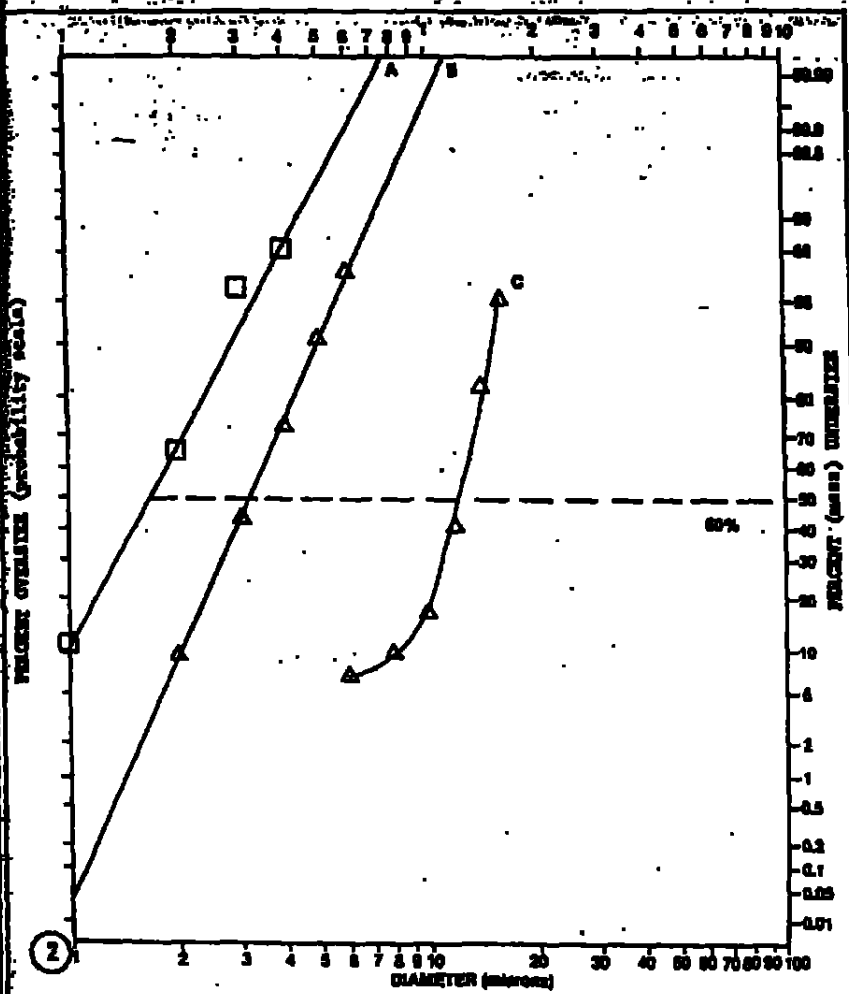


Fig 2.—Linear cumulative plot on probit scale representing particle size distribution of oxfendazole suspensions A, B, and C.

powders was determined by measuring the amount of gas adsorbed. Average surface area values and corresponding SD for the 3 raw material lots are included in Table 1. The Student's *t*-test (2-tailed) was used to compare these data.

Dissolution.—Dissolution-rate profiles of the 3 suspensions were determined at 37°C in 2,000 ml of water (pH 1.6) acidified with 5 ml of 37.4% HCl containing 0.5% polyacrylate 80,* at 30-rpm speed. The apparatus consisted of a 2,000-ml beaker immersed inside a controlled-temperature water bath maintained at 37°C ± 0.5°C. A dissolution drive assembly* equipped with stainless steel paddle stirrer was used. The rectangular paddle-blade dimensions were 75 mm × 25 mm, and the paddle was set at 2.5 cm above the beaker bottom. Preliminary experiments indicated that the dissolution of the suspension samples was too rapid to measure, particularly at

early time intervals at speeds greater than 30 rpm. A suspension sample (1 ml), equivalent to 22.55 mg of oxfendazole, was introduced into the surface of the dissolution medium. The stirrer switch was started at a previously set rate of 30 rpm. Sampling schedules for all the suspensions were set at 1, 3, 5, 7, 10, 15, and 30 minutes. These were established on the basis of the data obtained from the pilot trial run. At each time point, a 5-ml sample was withdrawn through a 10-ml disposable syringe from 1 cm below the surface and was replaced with the equivalent volume of drug-free dissolution medium. In preliminary trial runs, samples were withdrawn concurrently from various depth levels (1.0 to 10.0 cm range). Oxfendazole dissolution was observed to be uniform in the sampling region.

All samples were filtered through 0.45-μm nucleopore polycarbonate filters (25 mm) and absorbance measurements were made at 290 nm on a UV spectrophotometer.* The experiment was repeated 4

times with each suspension to establish the reproducibility of the results.

The dissolution data for the suspensions were treated by converting observed drug concentration at each sampling time to the amount dissolved and subsequently to the percentage dissolved. The mean percentage dissolved and SD values were computed and compared statistically at 7, 10, and 15-minute sampling times, using the Student's *t*-test for independent means (Table 1). Dissolution profiles for suspensions A, B, and C are shown in Figure 2.

Bioavailability in Sheep.—Twelve normal, young (8 to 12 months old) female Dorset sheep ranging in weight from 28.3 to 48.3 kg were used. During the entire study, they were fed a maintenance ration of chopped alfalfa hay. Water was available ad libitum. Feed was withheld overnight the day before dosing. The sheep were fed 6 hours after dosing on each of the 4-dose days. Each sheep was given each of the 3 suspensions according to a balanced Latin-square crossover design as shown in Table 2. (Parenthetically, suspension D, included in this part of the study, is unrelated to the 3 suspensions, and therefore will not be discussed in this report.) Oxfendazole was dosed at a rate of 5mg/kg of body weight. Suspensions were administered with 10-ml disposable syringes via Tygon stomach tubes (1.27 cm, OD × 0.83 cm, ID) passed into the rumen. After dose administration, syringes were rinsed with distilled water which was also passed through the stomach tube. Total volume of rinse water was 20 ml. An interval of 2 weeks separated each study.

Heparinized blood samples (10 ml) were obtained (jugular venipuncture) immediately before each dose (baseline sample) and at 1, 2, 4, 8, 12, 24, 48, 72, and 96 hours after dosing. Blood was centrifuged immediately, and plasma was transferred to appropriate test tubes and frozen. Oxfendazole was measured by the radioimmunoassay method of Nerenberg et al.¹¹ Mean oxfendazole plasma concentrations obtained for the 3 formulations are plotted in Figure 4 and are presented in Table 3 along with other related bioavailability values under consideration. Plasma value comparisons among the 3 oxfendazole formulations were made with respect to the following measurements: biological half-life ($t_{1/2}$); time to maximum plasma concentration (T_{max}); maximum plasma concentration (C_{max}); total area under the plasma concentration vs time curve (AUC); and observed plasma concentration values at all sampling times (Table 1). The $t_{1/2}$ was calculated by linear regression on the (log) plasma concentrations over the 48-through 96-hour time period. The AUC was estimated by the trapezoid rule up to 96 hours and thereafter by integration, using

* Pye Union Ltd, Cambridge, England.

TABLE 1—Relationship Among Particle Size, In Vitro Dissolution Pattern (Mean Percentage Dissolved $\pm$ se) and Plasma AUC of Three Oxfendazole Suspensions.

Suspension	Oxfendazole raw material particle size weight mean diameter (μ m)	Surface area (m^2/g)	Dissolution percentages* at various time intervals (min)			Plasma AUC† (μ g/0.1 ml $\times$ hr)
			T ₁	T ₁₀	T ₃₀	
A	1.83 [‡]	4.2912 $\pm$ 0.0043	91.9 $\pm$ 2.5	92.1 $\pm$ 1.3	97.3 $\pm$ 0.2	2932.00
B	8.30 [§]	2.0295 $\pm$ 0.0040	69.6 $\pm$ 7.5	81.6 $\pm$ 2.4	94.7 $\pm$ 2.5	2592.00
C	22.60 [¶]	2.1242 $\pm$ 0.0037	63.0 $\pm$ 4.5	82.5 $\pm$ 6.7	93.2 $\pm$ 11.7	2371.00

* At $P < 0.05$, (a) At all time points, suspension A was significantly different from suspension B and suspension C, and (b) suspension B was significantly different from suspension C at T₁ and T₁₀. † At $P < 0.05$, with respect to plasma AUC (total area under the plasma concentration vs T curve, μ g/ml $\times$ hr) values, suspension A was significantly different from suspension C. ‡ Micronized. § Milled. ¶ Coarse.

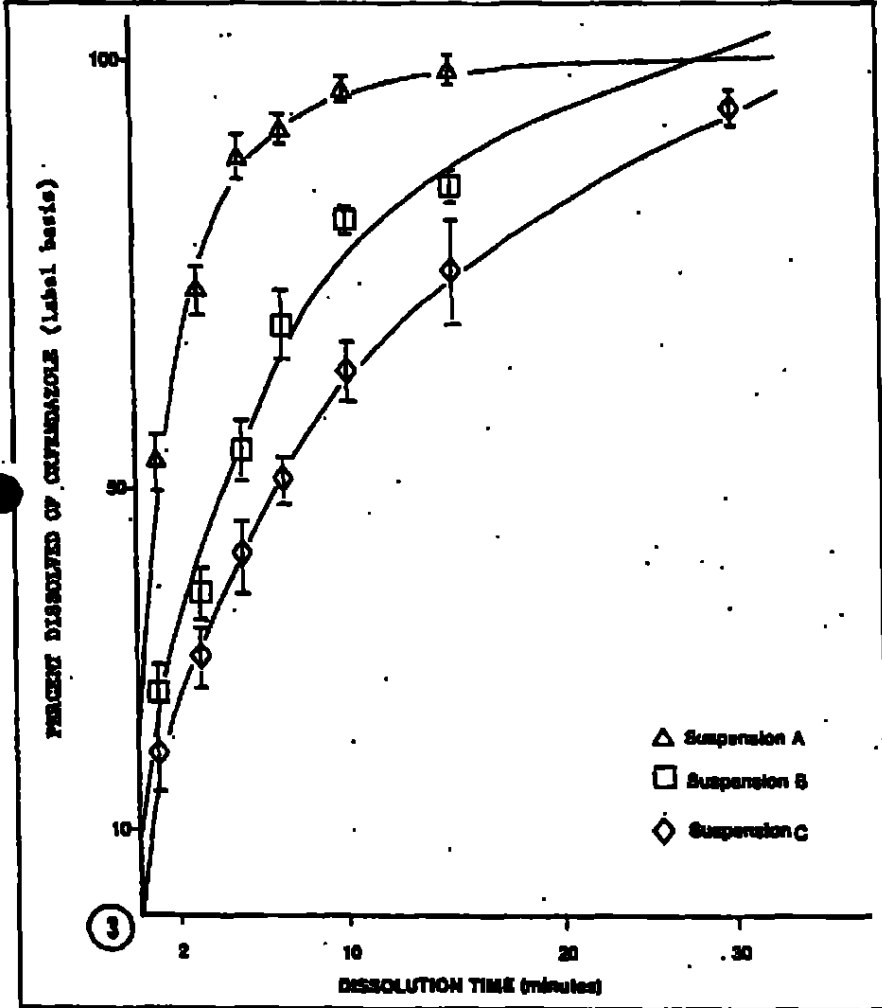


Fig 3—Comparison between dissolution rate of 3 oxfendazole suspensions.

the best fitting exponential decay curve for each sheep. Statistical analysis of these values was performed as pairwise comparisons, using Duncan's multiple range test at $P < 0.05$ (Table 4).

Anthelmintic Efficacy Study in Sheep—Twenty, 12-month-old, worm-free Marino weaner sheep were infected with 6,000 benzimidazole-resistant *H. contortus* larvae[‡] and were maintained in concrete

pens free of adventitious parasite infections. At 26 days after infection, 14 of these sheep were orally drenched with oxfendazole suspension at the rate of 5 mg/kg of body weight. The drug substance included in this suspension contained more than 90% of particles $< 10 \mu$ m in size. The remaining 6 animals acted as controls. Plasma oxfendazole concentrations were

[‡] NTZ-R, CIBRO Australia, NSW, Australia.

TABLE 2—Latin-Square Crossover Study Design for Oxfendazole Bioavailability Trial

Sheep No.	Period of study*			
	I	II	III	IV
1	D	B	A	C
2	B	A	C	D
3	A	C	D	B
4	C	D	B	A
5	D	B	A	C
6	B	A	C	D
7	A	C	D	B
8	C	D	B	A
9	D	B	A	C
10	B	A	C	D
11	A	C	D	B
12	C	D	B	A

* Period I = 1st dose administration; period II = 2nd dose administration, 3 weeks later; period III = 3rd dose administration, 4 weeks later; and period IV = 4th dose administration, 6 weeks later.

A = suspension A (micronized oxfendazole); B = suspension B (milled oxfendazole); C = suspension C (coarse oxfendazole); D = suspension D (experimental formulation unrelated to this study).

monitored in the drenched sheep for 34 hours after drug administration. Oxfendazole was measured by radioimmunoassay. The sheep were slaughtered on day 35 of the infection, and worm counts were made on individual sheep. The plasma AUC measurements were made at all the aforementioned times. Subsequent to slaughter of the sheep on day 35 of the infection, the anthelmintic efficacy of the oxfendazole in individual sheep was determined by comparison of worm counts with the geometric mean count in the 6 control sheep.

Results and Discussion

Histograms in Figure 4 indicated that the micronized material (lot A) was composed largely ($> 90\%$) of particles with diameters of $\leq 3 \mu$ m. The milled material (lot B) was intermediate between the micronized and the coarse materials (lot C). The dw values reflected this rank order (Fig 2; Table 1). Particle size distribution of lots A and B obeyed the log normal distribution function, as indicated by a linearity of the plot on the log probability graph in Figure 2. Lot C had a

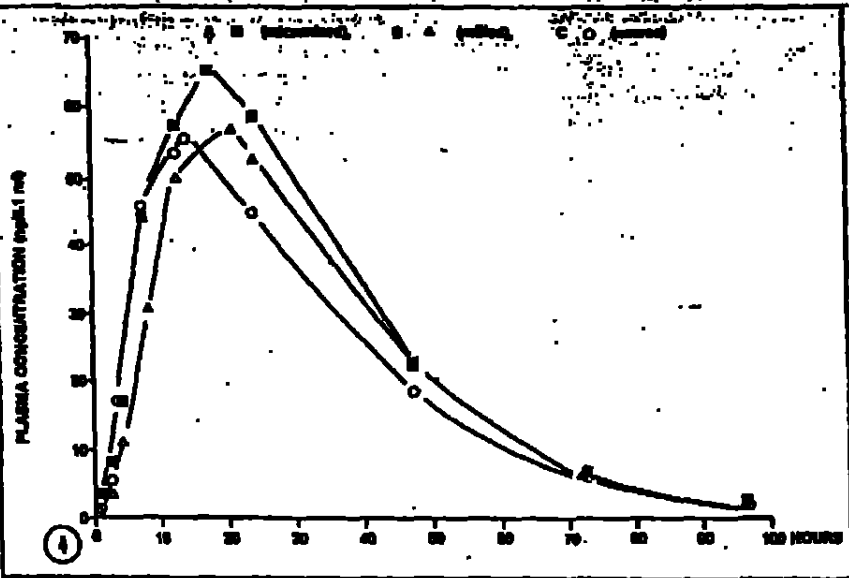


Fig 4—Mean oxfendazole plasma concentrations in sheep after an oral suspension dose (5 mg/kg of body weight) of 3 oxfendazole suspensions.

TABLE 3—Mean Plasma Concentrations and Related Bioavailability Values After a Single Oral Dose of Oxfendazole (5 mg/kg) of Three Sheep Suspension Formulations

Measurement	Formulation means ^a		
	Suspension A (micronized)	Suspension B (milled)	Suspension C (coarse)
Plasma concentration (µg/0.1 ml) at			
1 hr	2.77 (1.12)	0.80 (0.47)	1.19 (0.50)
2 hr	7.89 (3.91)	3.46 (1.35)	5.77 (3.30)
4 hr	16.90 (8.39)	10.20 (4.50)	10.90 (4.30)
8 hr	43.90 (11.84)	30.20 (8.00)	47.00 (12.30)
12 hr	57.20 (8.53)	48.50 (10.00)	53.20 (9.00)
24 hr	59.20 (10.30)	52.20 (15.20)	45.10 (12.20)
48 hr	23.20 (12.00)	22.00 (15.00)	14.00 (11.20)
72 hr	0.32 (5.00)	0.05 (4.70)	0.05 (3.70)
96 hr	1.83 (3.14)	1.83 (1.83)	1.83 (3.15)
<i>t</i> _{1/2} (hr)	12.30 (3.00)	12.70 (3.00)	12.90 (3.20)
<i>T</i> _{max} (hr)	12.00 (3.57)	21.00 (10.20)	14.00 (6.27)
<i>C</i> _{max} (µg/0.1 ml)	64.20 (14.57)	64.90 (12.15)	66.70 (10.42)
Total area (µg/0.1 ml/hr)	2,032.00 (305)	2,309.00 (706)	2,131.00 (702)

^a as in parentheses.
*t*_{1/2} = biological half-life; *T*_{max} = time to maximum plasma concentration; *C*_{max} = maximum plasma concentration.



Fig 6—A SEM micrograph of micronized sample (lot A). × 5,000.

TABLE 4—Pairwise Statistical Comparisons of Computed Measurements, Using Duncan's Multiple Range Test

Formulation comparison			
Measurement	A vs B	B vs C	A vs C
Plasma concentration at			
1 hr	S	NS	S
2 hr	S	NS	NS
4 hr	NS	NS	NS
8 hr	S	S	NS
12 hr	NS	NS	NS
24 hr	NS	NS	S
48 hr	NS	NS	NS
72 hr	NS	NS	NS
96 hr	NS	NS	NS
<i>t</i> _{1/2}			
	NS	NS	NS
	NS	S	NS
	NS	NS	NS
Total area	NS	NS	S

S = significant at *P* < 0.05, NS = not significant.

non-Gaussian or a skewed particle size distribution, and therefore, the log probability profile significantly deviated from linearity. Among the 3 lots, as expected, a decrease in *dw* of particles resulted in an increase in specific surface area. A significant difference was observed at *P* < 0.05 in the surface area values among the 3 lots A, B, and C (Table 1).

The Coulter counter data revealed a substantial difference in *dw* of particle size, 3.20 µm for lot B and 12.0 µm for lot C. However, surface area values did not show similar differences, eg, in lot C for a *dw* of 12 µm, the observed surface area value of 2.184 m²/g was excessively high; a value less than 1 m²/g would be more appropriately expected. The microscopic examination indicated that lot C contained many aggregates (15%) of finely divided primary particles (2 to 4 µm) in addition to numerous relatively larger primary particles (10 to 12 µm). In the SEM analysis, lot C had a more pronounced tendency to form aggregates and its micrographs appeared to confirm the presence of many smaller particles embedded in the aggregates (Fig 5-7). However, during Coulter counter measurements, these aggregates probably did not break up, even after sonication, thus reflecting a *dw* value of 12 µm. These observations may explain the apparent large disparity between *dw* and surface area values.

Analysis of variance was used to test the dissolution characteristics of oxfendazole suspensions prepared from the different raw material lots. The 3 groups were seen to be different from one another at *P* < 0.05. At *P* < 0.05 (at all time points) suspension



Fig 6—A SEM micrograph of milled sample (lot B). $\times 5,000$.

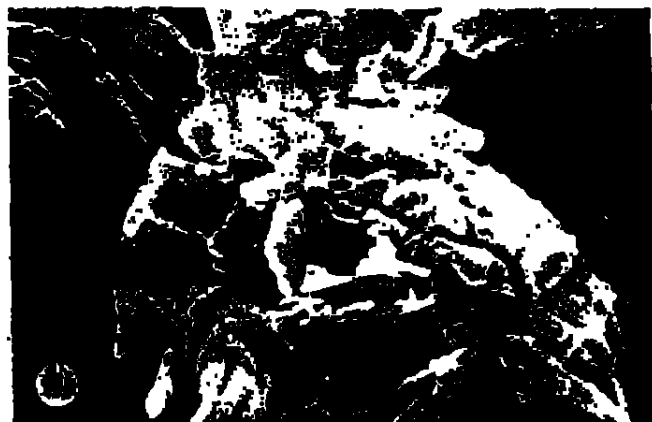


Fig 7—A SEM micrograph of coarse sample (lot C). $\times 5,000$.

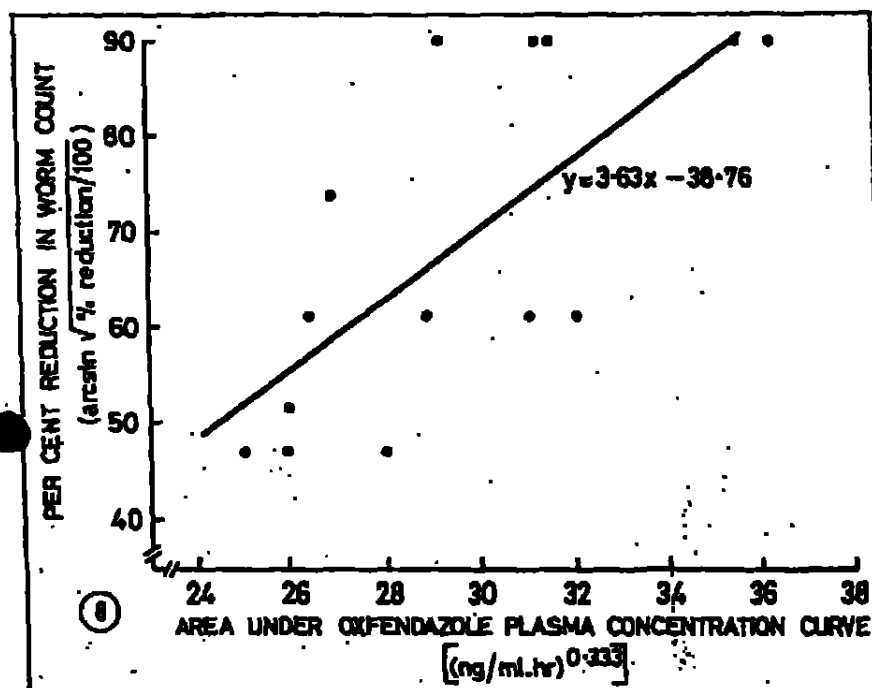


Fig 8—Linear regression of the reduction in count of *H. contortis*.

A was significantly different from suspension B and suspension C, and suspension B was significantly different from suspension C at T_1 and T_2 . In descending rank order, A, B, and C in that sequence, was observed. A preponderance of particles under $3 \mu\text{m}$ in lot A (96%) in comparison with those in lot B (44%) appeared to have resulted in dissolution-rate differences between them.

In view of small differences in the surface area values for lots B and C, the observed differences in their corresponding in vitro dissolution rates appeared to be surprisingly high. In the present instance, it seemed that the compact aggregates in suspension C did not sufficiently deaggregate during dissolution experiments, thus accounting for a slower dissolution rate. Parenthetically, preliminary pilot dissolution runs were performed to compare lots A, B, and C and the corresponding suspensions A, B, and C. In both sets, similar rank order differences were noticed.

In the sheep bioequivalence study, at 1 hour, the average plasma concentration observed after a dose of suspension A (micronized) was significantly higher than were those observed after suspension B (milled) and suspension C (coarse). At 8 hours, the average plasma value was lowest for suspension B and was significantly different from those for suspensions A and C. However, at 24 hours, plasma values for suspension C were lowest among the 3 suspensions. There was a significant difference between suspensions A and C. These differences disappeared during the subsequent time-point measurements.

The average peak C_{max} ranged from 557 ng/ml after suspension C to 643 ng/ml after suspension A. However, significant differences were not observed. Plasma values peaked earliest after suspension C (14.0 hours), and these were significantly different from suspension B (21.0 hours). The total AUC ranged from 21,210 ng/ml $\times$ hour for suspension C to 25,320 ng/ml $\times$ hour for suspension A; a statistical difference was detected between suspensions A and C. Therefore, suspension C was considered as significantly less bioavailable than was suspension A, whereas suspensions A and B were similar.

In the presence of ruminal fluid, the compact aggregates in suspension C appeared to have deaggregated into

smaller particles, which dissolved more rapidly. After dissolution, the dissolution rate in suspension A was significantly higher than in suspensions B and C.

The average plasma concentration from 12.3 to 12.5 hours were not of statistical significance. In the present study, the activity was highly with (0.01). This significant difference in the appropriate to the age and values were different at different sites.

Data (Table 1) indicate that the results in the present study were in agreement with the results of the previous study. The results of the present study were in agreement with the results of the previous study.

smaller primary particles, which rapidly dissolved to produce an early peak. After this, the larger particles appeared to have exhibited a dissolution rate limited absorption phenomenon, thus explaining smaller total AUC values in comparison with suspensions A and B.

The average biological $t_{1/2}$ ranged from 12.8 to 12.9 hours (mean 12.89 $\pm$ 0.25 sd). Significant differences were not observed in this regard.

In the Merino weaner sheep efficacy study, oxfendazole anthelmintic activity was found to correlate positively with AUC values ($r = 0.72$, $P < 0.01$). This relationship was shown as significant linear regression after appropriate transformations for percentage and cubic data (Fig 8). The AUC values were found to vary between different sheep over a 3-fold range.

Data (Table 1) indicated that particle size reduction of oxfendazole resulted in significant ($P < 0.05$) increase in surface area among the 3 raw material lots A, B, and C. This appears to explain the enhancement of dissolution rates observed in suspension A as compared with rates for suspensions B and C. In vitro dissolution data and in vivo sheep

plasma value data comparison similarly exhibited a rank-order correlation, particularly between suspensions A and C, thus indicating that oxfendazole's absorption, after its administration, is dissolution-rate limited.

In the Merino weaner sheep efficacy study, a correlation was found between oxfendazole's anthelmintic activity against *H. contortus* and plasma values when compared in individual sheep (Fig 8). This was further confirmed in a separate study by Anderson et al.⁴

Data indicate that substantial differences in particle size distribution of oxfendazole could influence its absorption characteristics. It should, therefore, be considered as an important measurement in considering oxfendazole efficacy against parasites.

⁴Anderson N, Lohy RH, Pritchard RH, et al: Controlled release of anthelmintic drugs: A new concept for prevention of helminthosis in sheep: Unpublished data, 1980.

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Table 6: Hyperlipemic test (5 weeks hyperlipemic diet, dosing last 14 days).

Code No.	Dose (mg/kg/day)	Cholesterol (mg%)				Triglyceride (mg%)			
		0	3	5	Difference ¹	0	3	5	Difference ²
K II	—	53.6 ± 8.3	50.4 ± 4.9	56.3 ± 8.9	—	69.9 ± 19.6	187.7 ± 28.7	99.3 ± 34.9	—
K II ³	—	—	78.8	118.6	—	—	181.9	204.6	—
ML 1073	300	—	112.0	183.4	(-/-)	—	163.8	208.8	(+/+)
ML 1077	300	—	117.0	115.2	(-/-)	—	198.4	238.2	(+/+)
LM 16	125	—	109.8	108.2	(0/-)	—	189.8	142.8*	(-/-)
K II	—	68.5 ± 10.8	124.7	181.4	—	46.2 ± 8.9	89.9	94.5	—
Cholestane	250	—	119.2	138.2*	(+/+)	—	58.7	106.4	(+/+)

¹ Control group (normal values after 0, 3 and 5 weeks; 0-values from 40 animals, all other values from 18 animals).
² Control group with hyperlipemic diet.
³ Signs in brackets show increase/decrease versus 3-week value (first line) or versus final value K II (second line).
* p<0.05.

Further supply of the thrombogenic diet combined with simultaneous dosing represents highest demands on efficacy of test compounds. Under these severe conditions active compounds show a slowing effect on hyperlipemia, while only highly active compounds produce a decrease of lipid level. From this point of view results manifest a high activity of ML 1073 and an inhibitory effect of ML 1077 on the cholesterol level. Triglycerides are only affected by LM 16, indeed with a surprising efficacy. Under these test criteria CPFB causes a high increase of both lipid fractions. The same inverted effect has been demonstrated in a previously published study [7]. In that test activity of etofibrate (220 mg/kg) and of a mixture of CPFB (150 mg/kg) and nicotinic acid (80 mg/kg) was investigated with a similar diet (pretreatment 21 days, dosing 7 days). Results indicate a rise by at least 30% of cholesterol and by at least 125% of triglyceride level under treatment. In spite of shorter experimental time and less severe conditions it can be stated

that both etofibrate and the mixture of CPFB and nicotinic acid were absolutely ineffective compared to our results. The different antilipemic action, compared to CPFB, and the possibly altered catabolism and/or uptake of exogenous lipids demand further investigation of the presented compounds in selected test designs.

5. Literature

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From Products Formulation Research Laboratory, Tanabe Sanyaku, Co., Ltd., Osaka (Japan)

Pharmacokinetics and Bioavailability of Diltiazem (CRD-401) in Dog

By K. Kohno, Y. Takeuchi, A. Etch, and K. Noda

Summary: Pharmacokinetic behaviour of a new coronary vasodilator, the *d*-cis-isomer of 3-acetoxy-2,5-dihydro-5-[2-(dimethylamino)ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one hydrochloride (diltiazem, CRD-401), as well as the bioavailability study of the release controlled tablet Herbasizer[®] have been described by using the proposed specific assay method of unchanged diltiazem in plasma.

Quantitative analysis after its oral administration evidenced that diltiazem was absorbed through the gastrointestinal tract in the intact form.

In the kinetic experiment, where diltiazem was administered *iv*, a rapid and large distribution of diltiazem into tissue compartment were suggested.

In the bioavailability study, a comparison of plasma concentrations of diltiazem between the two different crystals and the microencapsulated powder resulted in no difference in their bioavailability, when they were administered in the form of capsules.

In the single and multiple administration of the release controlled tablet, a slow and continuous absorption of diltiazem was observed. Elimination rate after the multiple dose regimen was in good agreement with that in a single dose, thereby indicating no accumulation in the body.

Zusammenfassung: Pharmakokinetik und Bioverfügbarkeit von Diltiazem (CRD-401) beim Hund

Die Pharmakokinetik eines neuen Koronar dilatators, des *d*-cis-Isomeren von 3-Acetoxy-2,5-dihydro-5-[2-(dimethylamino)-ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepin-4(5H)-on-hydrochlorid (Diltiazem, CRD-401), und die Untersuchung seiner Bioverfügbarkeit aus einer Tablette mit verzögerter Freigabe (Herbasizer[®]) wurden beschrieben unter Verwendung der vorgeschlagenen spezifischen Bestimmungsmethode für unverändertes Diltiazem im Plasma. Die quantitative Analyse zeigte nach oraler Verabreichung, daß Diltiazem im Gastrointestinaltrakt in unveränderter Form resorbiert wird.

Bei den kinetischen Untersuchungen, bei denen Diltiazem i.v. gegeben wurde, ergab sich eine rasche und umfangreiche Distribution im Gewebe-Kompartiment.
Bei den Untersuchungen zur Bioverfügbarkeit ergab sich beim Vergleich der Plasmakonzentrationen von Diltiazem zwischen zwei verschiedenen Kristallen und einem mikronisierten Pulver keine Differenz der Bioverfügbarkeit, wenn

die verschiedenen Formen in Kapseln verabreicht wurden. Bei einmaliger und mehrfacher Gabe der Tablette mit verzögerter Freigabe wurde eine langsame und gleichmäßig fortschreitende Resorption beobachtet. Die Eliminationsgeschwindigkeit nach wiederholter Dosierung stand in guter Übereinstimmung mit derjenigen nach einmaliger Gabe, woraus hervorgeht, daß im Körper keine Kumulation stattfindet.

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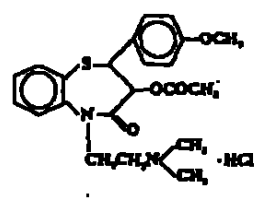
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1. Introduction

Diltiazem hydrochloride, d-cis-isomer of 3-acetoxy-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(p-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one hydrochloride. (CRD-401), is a new potent coronary vasodilator with additional hypotensive and negative chronotropic activities [1, 2]. Its clinical efficacy on angina [3, 4, 5] has been proved in administration of its release-controlled tablet (Herbesser®). Metabolic studies in mice [6] and rats [7] revealed its rapid absorption, distribution and elimination in the body. Deacetyl-O-demethyl-diltiazem, deacetyl-N,O-demethyl-methoxy-diltiazem and deacetyl-N,O-demethyl-diltiazem have been identified as major metabolites with minor pharmacological activity [1, 2].



In this paper, pharmacokinetic and biopharmaceutical properties of the release-controlled tablet were studied. Specific assay procedure was also described for determining the plasma level of unchanged diltiazem in dog.

2. Experimental
2.1. Dosage forms

Diltiazem hydrochloride exists as two different forms being designated by crystalline habit. Crystal I (prisms) and crystal II (plates), each of which has a particle size ranging from 400 to 500 μ and from 10 to 100 μ , respectively. These two crystalline forms and the powder obtained from crystal I with the particle size ranging from 5 to 30 μ were studied for their absorption characteristics. Aqueous solution of diltiazem hydrochloride (15–45 mg/ml) was administered as a standard formulation for determining the dose-bioavailability relationship. In single and multiple dose experiments, a tablet of 30 mg diltiazem hydrochloride (Herbesser) was used as a pharmaceutical preparation. The in vitro dissolution characteristics of diltiazem from this preparation was described in the latter section together with the plasma concentration data.

2.2. Dissolution rate test

Beaker method reported by Levy et al. [8] was used with a little modification to determine the dissolution rate for diltiazem. Dissolution media were solutions of pH 1.2 (the first fluid for disintegration test in I.P.D.), pH 5.5 (0.05 M acetate buffer solution) and pH 7.5 (the second fluid for the disintegration test in I.P.D.). A 500 ml beaker containing 400 ml of dissolution medium immersed in a constant temperature bath adjusted at 37°C. A four blade, 4-cm-diameter glass stirrer (1.7 cm wide) was placed in the center of the beaker and immersed to a depth of 3 cm from the bottom. The propeller was rotated with the speed of 35 rpm and a tablet was added into the solution. 5 ml of samples was withdrawn at certain time intervals and replaced by the same volume of fresh dissolution medium. The samples were filtered and analyzed spectrophotometrically for diltiazem at 237 nm.

*) Manufacturer: Tanabe Seiyaku Co. Ltd., Osaka (Japan).

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2.3. Determination of unchanged diltiazem in plasma

Quantitative analysis of diltiazem in plasma was carried out by using thin-layer chromatography (TLC). 1 or 2.5 ml of plasma samples was shaken with ether (15 ml $\times$ 3) for 5 min in a 50 ml polystyrene stoppered test tube. After centrifugation, the combined ether layer was evaporated to dryness in a tapered tube. The residue was dissolved in chloroform (70 μ l $\times$ 1, 50 μ l $\times$ 2) and was applied to TLC plate (Merck, Silica gel 60 F₂₅₄). Spots for unchanged diltiazem and the other soluble metabolites, which had been developed in the solvent system of chloroform/n-butanol/ethanol/methanol/acetic acid (8:3:3:1:1), were detected and analyzed at 237 nm by using a densitometer (Hitachi Two-Wavelength Double Beam Spectrophotometer 356, Thin Layer Chromatography Accessory). Reference wave length was set at 330 nm for this assay.

Fig. 1 is a standard calibration curve of diltiazem added into plasma. By this assay method, 0.02 μ g of diltiazem in 1 ml of plasma can be exactly determined.

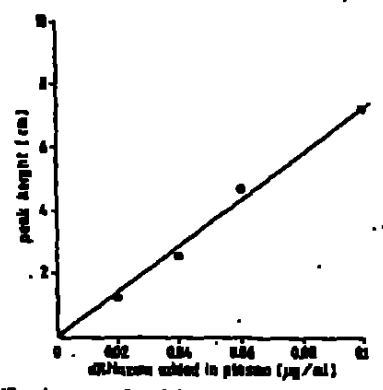


Fig. 1. Calibration curve for diltiazem added to plasma.

2.4. Intravenous administration

1 ml of diltiazem hydrochloride saline solution (1.5–5 mg/ml) was administered i.v. to two dogs via foreleg vein. The dog was fasted over night before the administration and kept fasting until the last sampling was done. Blood samples were taken just before the administration and 3, 6, 10, 20, 40, 60, 90, 120 and 240 min after the administration, and were immediately centrifuged to get the plasma samples for the determination of diltiazem.

2.5. Oral administration

In the administration of 30 mg diltiazem hydrochloride in the form of aqueous solution, three beagle dogs were orally given this dosage form and the blood samples were taken and processed for assay in the same way as described above.

In a comparative study of bioavailability between the two crystalline forms and the powder of diltiazem, six beagle dogs were orally given 60 mg of diltiazem hydrochloride in these three forms packed in the capsules with 20 ml of water in cross-over design. Blood samples were taken just before administration and 0.5, 1, 2, 3, 4, 5 and 7 h after the administration. Dogs were fasted as described above.

In order to evaluate the comparative bioavailability for these dosage forms, the area under the plasma concentration-time curve (AUC) from time zero to t_c was calculated by using trapezoidal rule. t_c is the time which corresponds to seven times the cal-

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where A_p and A_t are the fractions of total dose in central and peripheral compartments at time t after administration, respectively. Digital computer generated curves of the amount of diltiazem were shown in Fig. 4. The amount of diltiazem in the tissue compartment reached maximum value at 11 min after i.v. administration. At this time, the calculated ratios of the amount of diltiazem distributed into the central and tissue compartments were 13.4% and 56.9%, respectively. It is also apparent that diltiazem was almost eliminated from these two compartments within 7 h after administration.

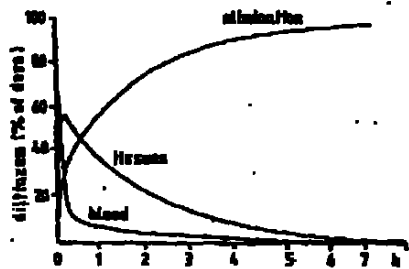


Fig. 4: Computed time course of distribution of diltiazem after i.v. administration of diltiazem hydrochloride according to two-compartment open model.

3.2. Bioavailability study

3.2.1. Dose-response relationship

Plasma concentrations following oral administration of a 30 mg dose in the form of aqueous solution showed that diltiazem appeared in plasma within 0.25 h and its mean maximum level was $11.0 \pm 1.3 \mu\text{g/dl}$, which was achieved 0.5 h after administration (Fig. 5).

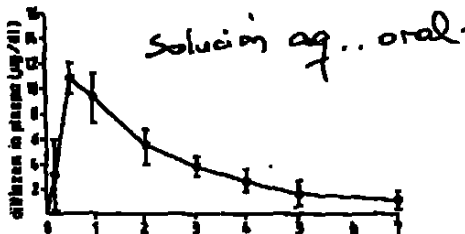


Fig. 5: Time course of plasma concentration of diltiazem following oral administration of 30 mg of diltiazem hydrochloride in the form of aqueous solution.

The linear relationship between the dose and AUC was confirmed over the range from 15 mg to 60 mg as shown in Fig. 6. This suggests that at least the range of the doses studied, AUC can be applicable in assessment of the bioavailability of diltiazem.

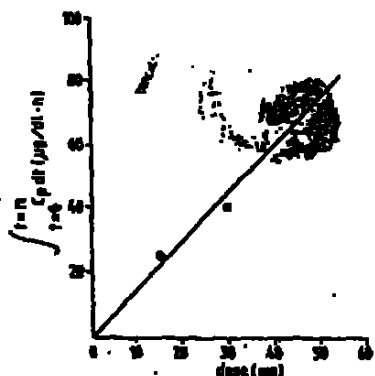


Fig. 6: Relationship between dose and AUC following oral administration of diltiazem hydrochloride in the form of aqueous solution.

3.2.2. Effect of crystalline form

The results of plasma concentration-time curve after oral administration of 60 mg diltiazem hydrochloride in three different forms of crystals, crystal I, crystal II and the micronized powder of crystal I, were represented in Fig. 7.

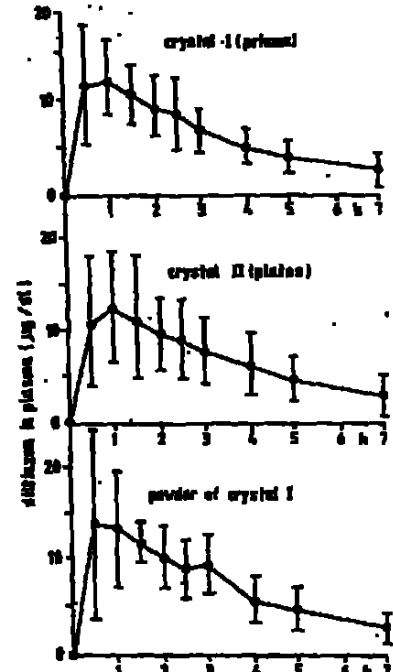


Fig. 7: Plasma concentrations of diltiazem following oral administration of 60 mg of diltiazem hydrochloride in three different forms of crystals.

The values of area under the plasma concentration-time curve (AUC), maximum plasma level (C_{pmax}) and the time to reach its peak (t_{max}) were summarized in Table 3. In crystal I, mean maximum plasma level was $16.2 \pm 3.7 \mu\text{g/dl}$, which was achieved in 1 h after administration. This peak level was comparable with that obtained after administration of crystal II. Whereas, the mean maximum plasma

Table 3: Bioavailability parameters for three different preparations of crystals following oral administration.

	Dose	C_{max} ($\mu\text{g/dl}$) AV	t_{max} (h) AV	AUC ($\mu\text{g/dl} \cdot \text{h}$) AV			
Crystal I	C	9.3	0.5	25.9			
	D	17.6	1.0	40.7			
	E	16.7	16.2	0.5	1.0	36.1	41.0
	F	17.0	±	2.5	48.1	±	
	G	20.3	1.7	0.5	49.8	29.0	
	H	16.2	1.0	45.4			
Crystal II	C	16.4	0.5	33.2			
	D	18.8	0.5	51.2			
	E	16.0	16.5	0.5	1.0	34.6	43.9
	F	17.1	±	1.8	48.4	±	
	G	19.6	1.9	2.5	32.6	16.6	
	H	16.2	1.5	75.6			
Powder of crystal I	C	15.9	0.5	37.0			
	D	14.9	1.0	36.8			
	E	18.3	17.6	1.0	1.0	24.2	46.4
	F	21.9	±	0.5	57.6	±	
	G	18.7	7.9	2.5	37.8	13.7	
	H	23.0	0.5	64.0			

level for powdered form of crystal I was $17.6 \pm 7.9 \mu\text{g/dl}$, and it appeared to be a little higher than those for two crystalline forms. However, no significant differences in the peak plasma levels were detected among all of the three dosage forms tested ($p > 0.1$). As for the bioavailability, these three test dosage forms produced almost the same area under the plasma concentration time curve, thereby no different bioavailability of diltiazem being suggested among these forms. This observation was further confirmed statistically by variance analysis ($p > 0.1$). From these findings, it is considered that diltiazem is little susceptible to influences from the crystalline forms in its bioavailability.

3.2.3. Single and multiple administration of release-controlled tablet

Dissolution rate curves of diltiazem from the release-controlled tablet were represented in Fig. 8. Dissolution of diltiazem was about 37% at 30 min, 55% at 60 min, and

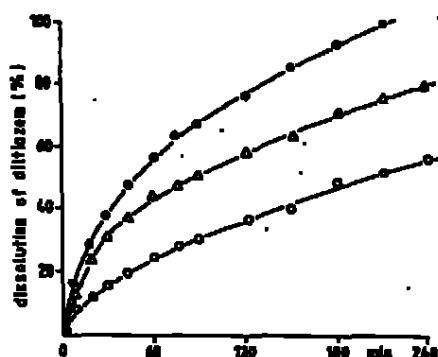


Fig. 8. Dissolution rate curves of diltiazem from the release-controlled tablet at different pH. $\circ$: pH 1.2; Δ : pH 5.5; $\square$: pH 7.4.

The results of plasma concentrations of 60 mg single and multiple doses of the release-controlled tablet were shown in Fig. 9. In the single dose administration, mean maximum plasma level was $8.6 \mu\text{g/dl}$ in two dogs. The time to reach its peak was 2 and 3 h after the administration. After reaching the maximum level, diltiazem in the plasma was gradually reduced, suggesting a slow and continuous absorption of diltiazem from the release-controlled tablet. During the multiple dose regimen, mean plasma level at 2.5 h after administration of the release-controlled tablet, where maximum steady state level would be attained, was $8.4 \pm 1.4 \mu\text{g/dl}$ in dog A and $6.5 \pm 2.0 \mu\text{g/dl}$ in dog B. Discontinuing the administration, plasma concentration fell down with the elimination rate which was in accord with that observed in the single dose administration. Therefore, it is proved that diltiazem is neither accumulated in the body nor influences its own elimination characteristics in the multiple dose regimen of the 60 mg t.i.d. of the release controlled tablet.

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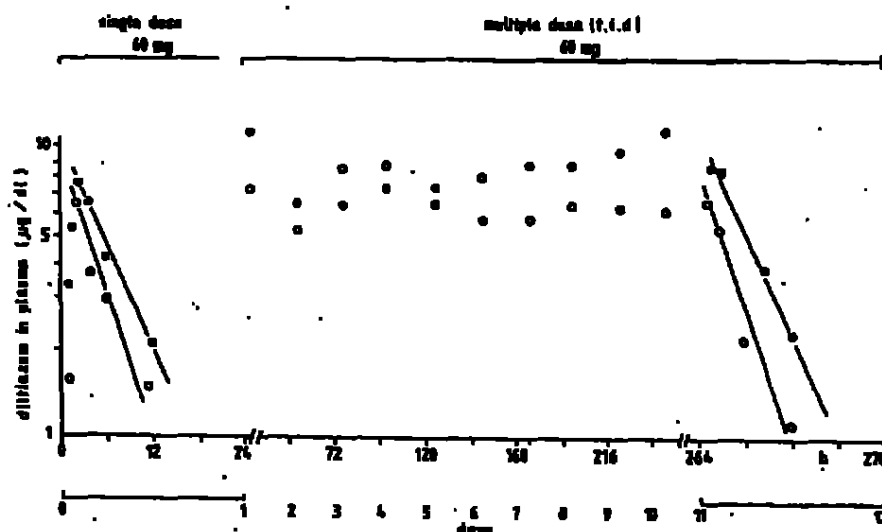


Fig. 9. Plasma levels of diltiazem after single and multiple administrations of the release-controlled tablet to two dogs.

95% at 180 min in the test fluid of pH 1.2 (simulated gastric fluid). With the increase of pH, the dissolution rate of diltiazem from the dosage form was gradually reduced. This seems to be a typical behaviour of diltiazem as a basic drug which is generally more soluble in the acidic than in the neutral and basic solvents.

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Impact of physical properties of formulations on bioavailability of active substance: current and novel drugs with cyclosporine

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Abstract

During the long period of cyclosporine-containing dosage forms development a lot of significant findings have been done especially in the field of drug delivery systems. Currently available drugs are based, from technological point of view, on self-dispersible drug delivery systems, which contain cyclosporine solved in pharmaceutically acceptable vehicles. One can find differences among particular systems: (a) at particle size distribution after dispersion; (b) at composition; and (c) at bioavailability of cyclosporine. As far as improvement of bioavailability between original brand leader formulation Sandimmune® and recent brand leader formulation Neoral® was followed by significant improvement in particle size distribution, it was generally assumed that the reason for this improvement have been found. Several other formulations, e.g. Coscopren® or SangCyA®-self-dispersible systems, more or less correspond with above-mentioned theory that smaller is better and by this principle bridged liquid based dosage forms with solid dosage forms. Bioavailability of novel drug delivery system which gives coarse dispersion with average particle size between 1–150 µm when dispersed have been tested on healthy volunteers. No difference among pharmacokinetic parameters of novel drug delivery system and microemulsion systems have been observed in spite of fact that average particle size of novel system is almost 1000× bigger.

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Keywords: Cyclosporine; Bioavailability; Drug delivery; Pharmacokinetics; Particle size distribution

1. Introduction

Cyclosporine containing dosage forms were introduced for use in transplantations two decades ago, and are the basis of most immunosuppressive protocols after solid organ transplantation (Kahan, 1989). Physico-chemical properties of cyclosporine does not allowed formulate only simple preparation for oral administration, but from the pharmaceutical point of view we should speak about drug delivery systems. Character of cyclosporine initiated step by step development of final dosage forms bearing in mind demands on safe, reproducible and cheap therapeutics with adequate patient compliance.

Soft gelatine capsules and oral solutions are widely used forms for oral administration, but this characteristic does not allowed to distinguish different behaviours of particular formulations, especially in term of bioavailability.

When one looks on cyclosporine dosage forms from historical perspective some general conclusions seems to be obvious. The first registered dosage form with cyclosporine (Sandimmune®, Novartis, Switzerland) was designed as an emulsion preconcentrate. Current brand leader formulation (Neoral®, Novartis, Switzerland) is in form of microemulsion preconcentrate and was launched on the market more

than 10 years after the first one. Significant improvement of treatment efficacy (Pollard et al., 1999) and bioavailability (Roessler et al., 1998) with Neoral® was reported. This improvement was connected with microemulsion character of administered drug (Hutchinson et al., 1996; Vanderscher and Melzer, 1994; Kovarik et al., 1996). Thus, the concept known from powder material that “smaller is better” was adopted also for liquid based dispersion systems.

Bearing in mind this situation bioequivalency study between new type of formulation and microemulsion type formulation was performed.

2. Experimental

Pilot bioequivalency study on 12 healthy volunteers (done in Phoenix Int. Life Sciences, Montreal) was aimed on comparison of bioavailability of two different formulation in form of soft gelatine capsule containing 100 mg of cyclosporine (formulations: A, GEM101; and B, GEM304); giving dispersion within the range of 1–150 µm with Neoral® 100 mg capsules (formulation C). Consequently, visualisation of novel drug delivery system and precise evaluation of particle size distribution were done.



Fig. 1. Photomicrograph of gel based emulsion (GBE).

Based on the visual observation the novel system was denominated as gel based emulsion (GBE). Its photomicrograph is shown in Fig. 1.

2.1. Bioequivalence study design

This open-label, randomised three-period crossover study was designed for 12 Caucasian healthy male volunteers, within 18–45 year of age and body weight $\pm 10\%$ of ideal one. The test medications and the reference medication were to be administered in randomised sequence as single oral doses under the fasted condition. Each dose contained 200 mg cyclosporine (two capsules of 100 mg). The duration of the washout period between treatments was to be at least 7 days. In each study period, 14 blood samples were to be taken before administration and 20, 40, 60 min, and 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12 and 24 h after administration. Adverse events were to be monitored during the entire study. Blood was to be taken from the antecubital vein into EDTA plastic tubes (Sarstedt Monovettes). The samples were to be deep-frozen (-20°C).

Cyclosporine whole blood concentrations were determined by means of a specific RIA.

$\text{AUC}_{(0-\infty)}$ and C_{max} were defined as the primary variables for the evaluation of bioavailability. $\text{AUC}_{(0-24)}$, t_{max} , $t_{1/2}$, $\text{MRT}_{(0-24)}$, $\text{MRT}_{(0-\infty)}$, and Frel were secondary variables.

From the concentration–time data of the parent compound, the pharmacokinetic parameters were to be deter-

mined for each individual dataset by means of non-compartmental analysis using TopFit 2.0.

C_{max} and t_{max} were to be taken directly from the observed concentration–time data. The elimination rate constant (k_{el}) was to be calculated by log-linear least squares regression analysis of the terminal part of the plasma concentration–time curve. The area under the concentration–time curve ($\text{AUC}_{(0-\infty)}$) was to be calculated up to the last measurable concentration–time point (t) by the linear trapezoidal rule. Extrapolation to infinity ($\text{AUC}_{(0-\infty)}$, $\text{AUC}_{(0-\infty)}$) was to be done by dividing the last observed concentration by the elimination rate constant.

2.2. Statistical analysis

Arithmetic means, standard deviations, and medians were to be calculated for concentration–time data.

For t_{max} and $t_{1/2}$, arithmetic means, standard deviations, and 95%-confidence intervals for means were to be calculated. For C_{max} , $\text{AUC}_{(0-24)}$, $\text{AUC}_{(0-\infty)}$, and F_{rel} , geometric means, standard deviations, and 95%-confidence intervals for means were to be calculated.

The primary variables $\text{AUC}_{(0-\infty)}$ and C_{max} , after logarithmic transformation, were to be subjected to an ANOVA with the factors subject, formulation and period. For the evaluation of bioequivalence, 90%-confidence intervals were to be calculated. The accepted bioequivalence ranges were (0.80–1.25) for AUCs and (0.70–1.43) for C_{max} , respectively.

Table 1
Summary of pharmacokinetic data

Parameter	$t_{1/2}$ (h)	t_{max} (h)	C_{max} (ng/ml)	AUC _(0–t) (ng h/ml)	AUC _(0–∞) (ng h/ml)
Formulation A					
Arithmetic mean	6.24	1.33	1572.69	4631.73	4861.85
S.D.	1.3	0.33	351.28	1204.56	1261.87
Geometric mean	6.12	1.3	1329.84	4483.35	4712.35
Minimum	4.08	1	908.1	2633.32	2873.57
Maximum	8.24	2	1930.3	6432.76	6694.33
Formulation B					
Arithmetic mean	6.41	1.3	1196.49	4438.33	4696.56
S.D.	1.3	0.48	308.26	1082.91	1143.13
Geometric mean	6.28	1.43	1167.84	4326.15	4576.94
Minimum	4.21	1	831.8	3130.66	3254.08
Maximum	8.93	2.3	1785	6206.13	6643.13
Formulation C (reference)					
Arithmetic mean	6.13	1.33	1538.35	4667.21	4867.33
S.D.	1.32	0.33	360.35	1238.41	1430.5
Geometric mean	5.99	1.3	1307.19	4472.8	4706.33
Minimum	3.92	1	820.7	2933.67	3028.58
Maximum	7.87	2	1803.3	7230.08	7686.89

Table 2
Bioequivalence criteria

		Lower 90% confidence limit	Point estimate	Upper 90% confidence limit
AUC _(0–∞)	Form A/reference	0.93	1.00	1.08
	Form B/reference	0.88	0.97	1.07
AUC _(0–t)	Form A/reference	0.93	1.00	1.09
	Form B/reference	0.87	0.97	1.07
C_{max}	Form A/reference	0.90	1.02	1.16
	Form B/reference	0.79	0.99	1.01

2.3. Pharmacokinetics and bioequivalency data

Summary table of pharmacokinetic data is shown in the Table 1.

The ANOVAs indicated a statistically significant medication effect for all three variables and a significant period effect for AUC_(0–∞) and AUC_(0–t) ($P < 0.05$). For the AUC- and C_{max} -ratios, the Table 2 shows confidence intervals (CI) and point estimates.

2.4. Particle size evaluation

Masterizer Micro, version 2.18 (Malvern Instruments Ltd.), evaluated particle size distributions of novel GEM formulations. Fig. 2 shows histograms of particle size distribution of formulation A (GEM101) and formulation B (GEM304). Effective diameter of formulation A (resp. B) deduced from histogram was 92.05 μm (36.23 μm).

3. Discussion

This study was performed in a population of young healthy males (19–39 years) according to the Clinical Study

Protocol. The study medications appeared to be safe and well tolerated.

This clinical study showed that formulations A and B meet criteria for bioequivalency with reference formulation C.

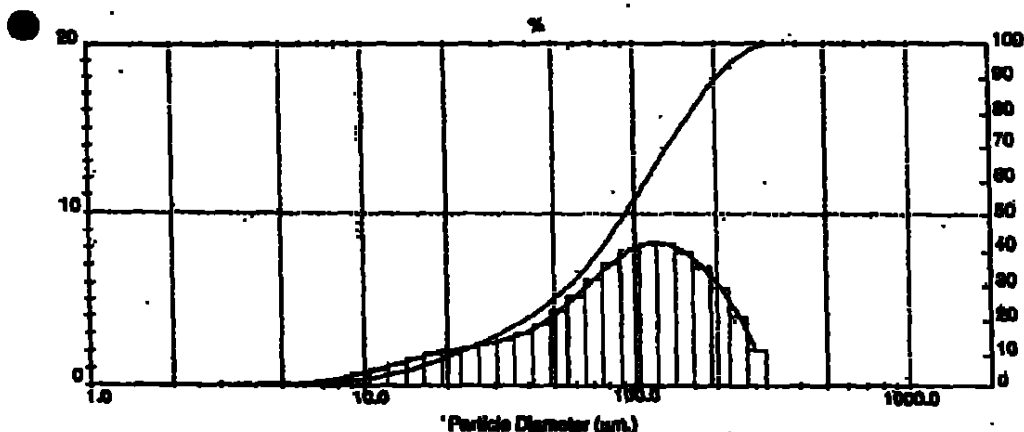
Comparison of effective size of particles created after dispersion on the other hand showed that formulations A and B give approximately 1000 times larger particles than reference formulation C.

These findings interfere with previous concept that smaller is better.

Nevertheless, improvements in bioavailability which consequent on better dispersion abilities besides to Neoral® (e.g. giving smaller particles after administration) was described with Conaspor® (emulsion giving formulation; Galema, Czech Republic; Noble and Markham, 1995) and SangCys® (microdispersion giving formulation; SangStat, USA; Duchin, 1997; Canafix et al., 1999). Summary of average particle size of mentioned cyclosporine containing formulations is given in the Table 3 (adopted) (Andrysek, 2000).

Meantime, biofarmaceutical experiments focused on correlation between particle size distribution and bioavailability on dogs were conducted. Those experiments showed that the same improved bioavailability is achievable with

(a) formulation A- Gem 101



Malvern Instruments Ltd.

Malvern, UK

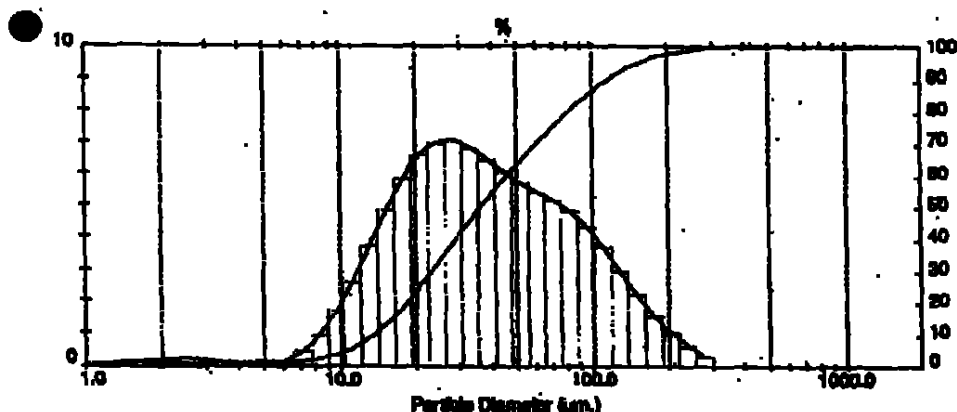
Tel: +44 (0)1684-892455 Fax: +44 (0)1684-892788

MasterSizer Micro Ver. 2.18

Serial Number: 34044-02

12 May 99

(b) formulation B- Gem 304



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12 May 99 12:30

Fig. 2. Histogram of particle size distribution: (a) formulation A, GEM101; (b) formulation B, GEM304.

systems giving particle size distribution in near submicron range (Andrysek et al., 1998). Consequently, no correlation between particle size distribution resp. molecular weight of aggregation and bioavailability was observed when model microdispersed systems with 141 nm (309 and 1001 nm) average size of particles were evaluated (Andrysek and Piatrlik, 1999).

Based on current findings it is possible to deduce that particle size distribution is not the crucial point for bioavailability of hydrophobic drugs like cyclosporine from solution

based systems. The reason for good bioavailability of cyclosporine form GEM could be seen in two different factors:

1. *Physical*—in spite the fact that GEM dispersion is rather coarse it has different properties comparing to emulsion; the physical reason for that could be seen in ability to overcome unstirred water layer in the lumen of a small intestine and adhere into the intestinal wall—thus could be ensure sufficient time and concentration gradient for absorption of cyclosporine.

Table 3
Effective diameter of dispersed particles (cyclosporine containing drugs)

	Eff. diameter (µm)	CV%
Sandimmune®	3.73 ^a	61.3
Cancrospan®	1.73 ^a	22.3
Cyclosporine cps (RON Lab.)	0.99 ^a	24.7
Congrat® cps	0.84 ^a	19.5
SangCya®	0.09 ^b	15.1
Neoral®	0.83 ^b	12.9

Eff. diameter, effective diameter; CV%, coefficient of variation (%).
^a Optical microscope technique; computer analysis by Lucid®. Laboratory Imaging Inc.
^b Dynamic laser scattering performed on BI 2002SM, BI9000 AT digital correlator (Brookhaven Inc.) with argon 514 nm laser (Laser Laser Inc.).

2. **Metabolism**—all mentioned drugs and formulation with cyclosporine contain non-ionic surfactant which probably can affect permeation or absorption of cyclosporine through the intestinal wall.

The effects of excipients on drug bioavailability have been documented since the early 1990s. It was described that surfactants can cause reversible microvillar local damage and that way increase permeability through the intestinal wall (Swenson et al., 1994). Disruption of the cellular tight junction is another potential effect of excipients observed on Caco-2 cells model (Neruckar et al., 1996). More likely than effect on tight junction the effect of excipients modified activity of multi-drug resistance protein—efflux transporter, MDR 1, also called P-glycoprotein (P-gp.) or metabolic enzymes (cytochrome P450 subclass 3A4; CYP3A4; Hingger et al., 1999). Not only surfactants but also polyethylene glycol 300 was reported as MDR 1 inhibitor with effect on membrane fluidity.

CYP3A4 activity is also modified by excipients. Different level of suppression of its activity was reported in accordance with application of Cremophor RH 40, lecithin–taurocholate admixture, Tween 20 and Vitamin E TPGS (Mountfield et al., 2000).

4. Conclusion

Novel gel based emulsion giving drug delivery system for hydrophobic drugs (GEM; GEM DDS) was devel-

oped and tested. In spite the fact that dispersion is not as good as in microemulsion pre-concentrates and effective diameter of created particles meets almost 100 µm size, bioequivalency with microemulsion preparation was proved.

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Short communication

Nanospheres of cyclosporin A: poor oral absorption in dogs

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Abstract

The cyclic undecapeptide cyclosporin A (CYA) used as first-line therapy in the prevention of xenograft rejection following organ transplantation, is extremely hydrophobic. Marketed formulations employ solubilising agents to facilitate absorption in the gastrointestinal tract. In this study, cyclosporin A nanospheres were prepared by precipitation in an aqueous surfactant solution. The particle matrix consists of the drug itself. Drug was dissolved in acetone and mixed rapidly with an aqueous solution of polysorbate 80 and sodium dodecyl sulphate (SDS). The acetone was evaporated to give a colloidal precipitate of spherical particles. Particle size could be controlled by varying the quantity of starting materials to give nanospheres of Z-average diameters in the range 250–900 nm with low polydispersity. The oral absorption of CYA from these nanospheres was compared to absorption from a microemulsion formulation in the dog. The relative bioavailability of cyclosporin A from nanospheres was only 3%, based on comparison of the area under the blood concentration–time curve (AUC) values for the two formulations. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Cyclosporin A; Nanospheres; Oral absorption; Dogs

The formulation of the immunosuppressant drug cyclosporin A (CYA) for oral use presents a considerable challenge as it is practically insoluble in water (Imailos et al., 1991). It is generally

accepted that it cannot be absorbed following oral administration unless it is first solubilised in gastro-intestinal (GI) fluids by appropriate excipients. In this study the absorption of CYA from nanospheres comprising only the drug itself was investigated. Such particles might be absorbed intact across the gut wall and avoid pre-systemic dissolution limitations.

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Extremely hydrophobic drugs such as CYA can be made to form such particles using a precipitation technique as reported by Gafmann et al. (1994). Drug is dissolved in an organic solvent and mixed rapidly with an aqueous solution of surfactant stabilisers to give a colloidal precipitate of spherical particles. CYA nanospheres were produced using a similar technique. The absorption characteristics of CYA from the colloidal precipitate were assessed in dogs and compared to those of the Neoral® microemulsion formulation.

Particle size has been shown to be an important factor in the extent of absorption of submicron polymeric spheres across the gut wall (Florence and Jani, 1993). Attempts were therefore made to control the diameter of the CYA nanospheres by varying production conditions.

CYA (purity 99.6%) was obtained from Galena (Czech Republic). All organic solvents used were high performance liquid chromatography (HPLC) grade and purchased from Fisher (UK). Polysorbate 80 was obtained from Fluka (UK) and sodium dodecyl sulphate (SDS) from BDH (UK). Double de-ionised water was used throughout. Neoral® was obtained from Sandoz (UK).

CYA nanospheres were prepared by quickly adding a solution of CYA in acetone through 4 ml of an aqueous surfactant solution in a glass vial via a micropipette. A non-ionic (polysorbate 80) and an ionic surfactant (SDS) were used to stabilise nanospheres by steric and electrostatic mechanisms, respectively. The surfactant solution was stirred rapidly using a magnetic bar during addition of the CYA solution. Acetone was evaporated under vacuum at 60°C in a rotary evaporator at constant speed for 5 min then under a steady stream of nitrogen for 2 min. Vacuum pressure, rotary speed, nitrogen flow-rate and SDS concentration (0.01% w/v) were kept constant for each batch of nanospheres produced.

The quantities of either CYA, polysorbate 80 or acetone were varied in turn to investigate the influence of these components upon particle diameter (formulations A–G; Table 1). Where the quantity of acetone was increased to 1 ml, the time of evaporation was increased to 8 min to ensure complete removal of the solvent.

The morphology of CYA nanospheres were assessed by scanning electron microscopy (SEM). A small quantity of the nanosphere suspension was dried and put on to an SEM stub using carbon adhesive discs and transferred to an Emitech K550 sputter-coater. The sample was gold-coated for 3 min at 30 mA and then viewed and photographed under a Philips XL20 scanning electron microscope.

The Z-average diameter and polydispersity of each batch of CYA nanospheres was determined by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, Malvern, UK) equipped with a fixed angle 10 mW helium–neon laser operated at 633 nm. Particle size was measured immediately after production and also after storage at room temperature for 24 h.

The oral absorption of CYA from nanospheres produced as described above was compared to that of Neoral® in two groups of five to ten male beagle dogs (11–16 kg). Each formulation was diluted to a constant volume of 5 ml with water immediately before administration and given at a dosage of 100 mg CYA/dog as a single oral dose. The nanospheres were made using 50 mg polysorbate 80 and 200 µg SDS/dose and had a Z-average diameter of 394 ± 35 nm. Serial blood samples (2 ml) were then taken from the antecubital vein up to 24 h post-dose and mixed with ethylene-diamine tetra-acetic acid as anticoagulant. A polyclonal antibody radioimmunoassay kit was used to determine CYA concentrations in

Table 1
Quantities of starting materials used in nanosphere formulations^a

Formulation	CYA (mg)	Acetone (ml)	Polysorbate 80 (% w/v)
A	15	0.5	1.5
B	25	0.5	1.5
C	75	0.5	1.5
D	25	0.25	1.5
E	25	1	1.5
F	25	0.5	0.5
G	25	0.5	2.5

^a Sodium dodecyl sulphate (SDS) was included in all formulations at 0.01% w/v. The volume of aqueous phase was also kept constant at 4 ml.

whole blood (Immunotech, Czech Republic). The bioavailability of each formulation was compared by calculating the area under the blood concentration-time curve (AUC) using the log-trapezoidal method. The maximum blood concentration (C_{max}) reached and the time take to achieve C_{max} (T_{max}) were also determined for CYA nanospheres and the Neoral® microemulsion.

SEM revealed that CYA precipitated as submicron spherical particles using the above method. When the quantities of starting were varied in turn independently, PCS indicated that the quantity of CYA and the volume ratio of the organic phase to the aqueous phase were the chief determinants of particle size (Fig. 1). Fig. 2 shows SEM micrographs of CYA nanospheres produced using different component proportions to give nanospheres of diameter 352 and 824 nm as determined by PCS. The latter were produced by simultaneously increasing the volume of acetone and quantity of CYA used. Within the range of quantities of each component used it was found that the polysorbate 80 concentration could be varied between 0.5 and 2.5% w/v with little effect upon size. Polydispersity ranged from 0.1 to 0.5 and tended to increase slightly upon storage. However, changing the component quantities did not result in any trend in this parameter (Fig. 1). In all batches of nanospheres size increased rapidly in the 24 h period following production (11-32% increase).

Oral absorption studies in dogs showed that CYA could be absorbed from nanospheres but the extent of absorption was markedly less than Neoral®. CYA bioavailability from the nanosphere formulation was only 3%, based on comparison of AUC values for nanospheres and Neoral®. The maximum concentrations achieved also showed considerable variability compared to Neoral® (Table 2). CYA blood concentrations from the nanosphere suspension declined slowly until approximately 11 h post-dose, when a secondary rise in blood concentration was seen. This may have been due to delayed translocation of CYA into the bloodstream from nanosphere 'depots' in the intestinal mucosa or the mesenteric lymphatic system. The low bioavailability seen might also be due to the anionic charge conferred

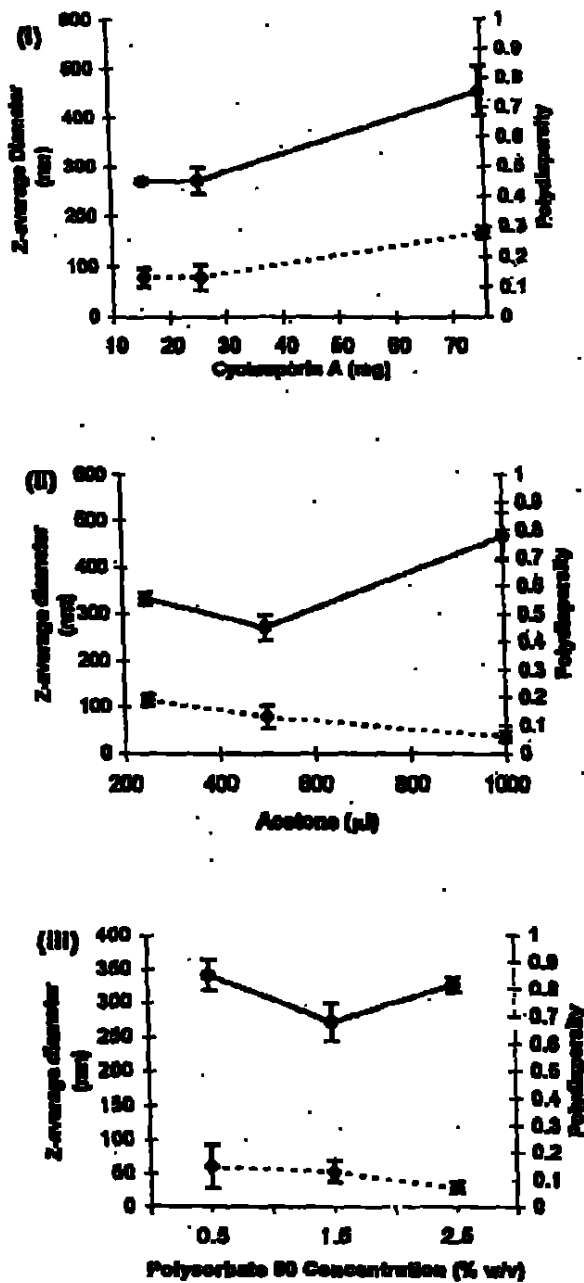


Fig. 1. Effect of varying (i) cyclosporin A (CYA) quantity, (ii) acetone volume, and (iii) polysorbate 80 concentration upon particle size (●) and polydispersity (◆) of CYA nanospheres. Each data point represents the mean of three batches with standard deviations shown.

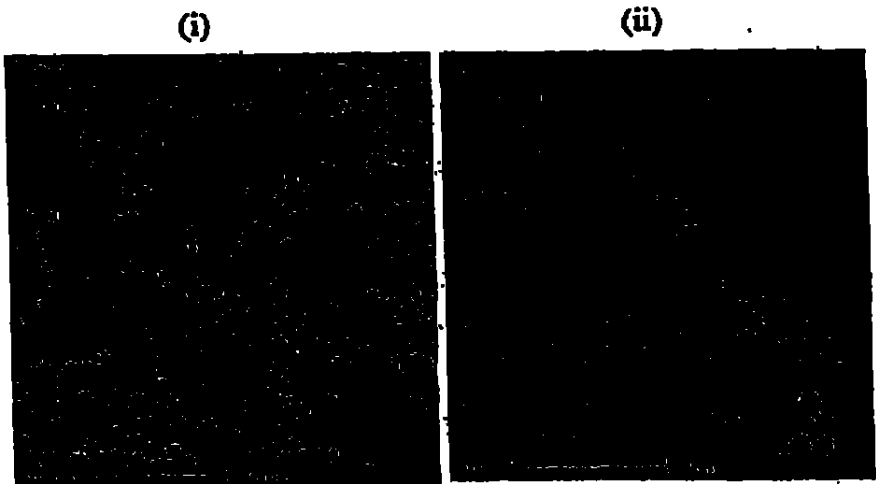


Fig. 2. Scanning electron micrographs of cyclosporin A (CYA) nanospheres with mean Z-average diameters of (i) 352 nm and (ii) 824 nm.

Table 2
Pharmacokinetic parameters for cyclosporin A (CYA) nanospheres and Neoral[®] given to dogs as a single oral dose of 100 mg CYA^a

Formulation	AUC (ng h/ml)	Initial C _{max} (ng/ml)	T _{max} (h)
CYA nanospheres	351 (20%) ^b	25 (38%) ^b	2.2 (36%) ^b
Neoral [®] microemulsion	12 790 (16%) ^c	1689 (26%) ^c	1.9 (37%) ^c

^a Relative standard deviations are shown in brackets.

^b n = 5.

^c n = 10.

to the particles by the SDS used in the formulation, since association of the sphere to the negatively-charged surface of the intestinal mucosa might be hindered.

In conclusion, therefore, CYA can easily be formulated as nanospheres even in the absence of particle-forming polymers. Particle size can be controlled simply by changing the quantity of the drug itself and other components used in the production of such nanospheres. However, the ability of this system to facilitate oral absorption of CYA in dogs was poor compared to an efficiently-absorbed microemulsion formulation.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

439

DIVISION DE NUEVAS CREACIONES

/2020

Bogotá, D.C., Agosto 29 de 2006

Expediente No.: 01 087 299

Título de la solicitud:

"Composiciones Inhibidoras de Ciclooxigenasa-2
que tiene rápido acceso de efecto terapéutico".

Anterioridades D1- D4

[54] **PHENYL HETEROCYCLES AS COX-2
INHIBITORS**

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[73] Assignee: Marck Frost Canada, Inc., Kirkland, Canada

[21] Appl. No.: 179,467

[22] Filed: Jan. 10, 1994

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 82,196, Jan. 24, 1993, abandoned.

[51] Int. Cl.⁶ A61K 31/53; C07D 307/02

[52] U.S. Cl. 514/241; 514/242; 514/252; 514/267; 514/359; 514/362; 514/363; 514/365; 514/372; 514/374; 514/378; 514/383; 514/451; 514/444; 514/473; 514/499; 514/461; 549/60; 549/295; 549/323; 549/218; 549/222; 544/180; 544/238; 544/333; 544/374; 548/127; 548/128; 548/131; 548/134; 548/136; 548/125; 548/143; 548/202; 548/206; 548/235; 548/247; 548/365.7; 548/255; 548/262.5

[58] Field of Search 549/218, 222, 549/295, 60, 323; 514/99, 451, 461, 241, 242, 252, 267, 359, 374, 378, 383, 444; 544/180, 238, 333, 374; 548/127, 128, 131, 134, 125, 136, 143, 202, 206, 235, 247, 365.7, 255, 262.5

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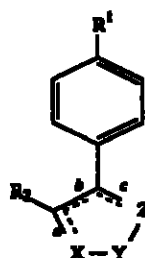
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Attorney, Agent, or Firm—Curtis C. Panzer, David L. Rose

[57] **ABSTRACT**

The invention encompasses the novel compound of Formula I useful in the treatment of cyclooxygenase-2 mediated diseases,



Papaoxi

The invention also encompasses certain pharmaceutical compositions for treatment of cyclooxygenase-2 mediated diseases comprising compounds of Formula I.

25 Claims, No Drawings

27

28

TABLE I-continued

TABLE I-continued

	Example	Method
	20	I
	21	I
	22	I
	23	I
	24	I
	25	I

lofeconib

	Example	Method
	26	I
	27	I
	28	I
	29	I
	30	I
	31	I

(11) Patent Number: 5,633,272

[45] Date of Patent: May 27, 1997

benzenesulfonida

[54] SUBSTITUTED ISOXAZOLES FOR THE TREATMENT OF INFLAMMATION

94/17029	2/1994	WIPO .
94/20475	2/1994	WIPO .
95/00501	1/1995	WIPO .
95/12587	5/1995	WIPO .
95/14672	6/1995	WIPO .

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by Kathy L. Rogers, legal
representative; Karen Seibert, 11930
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Primary Examiner—Johann Richter
Assistant Examiner—Laura L. Stockton
Attorney Agent or Firm—Joseph W. Bullock

[57] ABSTRACT

A class of substituted isoxazolyl compounds is described for use in treating inflammation and inflammation-related disorders. Compounds of particular interest are defined by Formula II

1211 Appl. No.: 673,824

[22] Filed: Jan. 7, 1995

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 387,680, Feb. 13, 1993,
abandoned.

[51] Int. Cl.⁶ C07D 261/06; C07D 261/10;
C07D 261/12; C07D 261/14; A61K 31/42

[52] U.S. Cl. _____ 514/378; 548/182; 548/186;
548/190; 548/193; 548/202; 548/203; 548/225;
548/228; 548/229; 548/232; 548/234; 548/235;
548/243; 548/245; 548/247; 548/248; 546/272.1;
544/403; 514/255; 514/340; 514/365; 514/369;
514/370; 514/374; 514/376; 514/377; 514/380

[58] Field of Search 548/247, 243,
548/245, 249; 514/380, 378

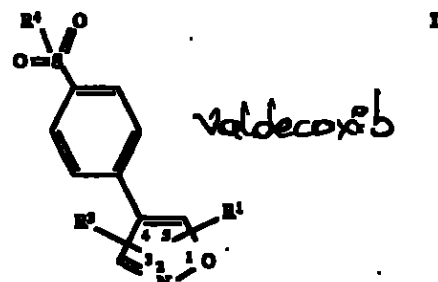
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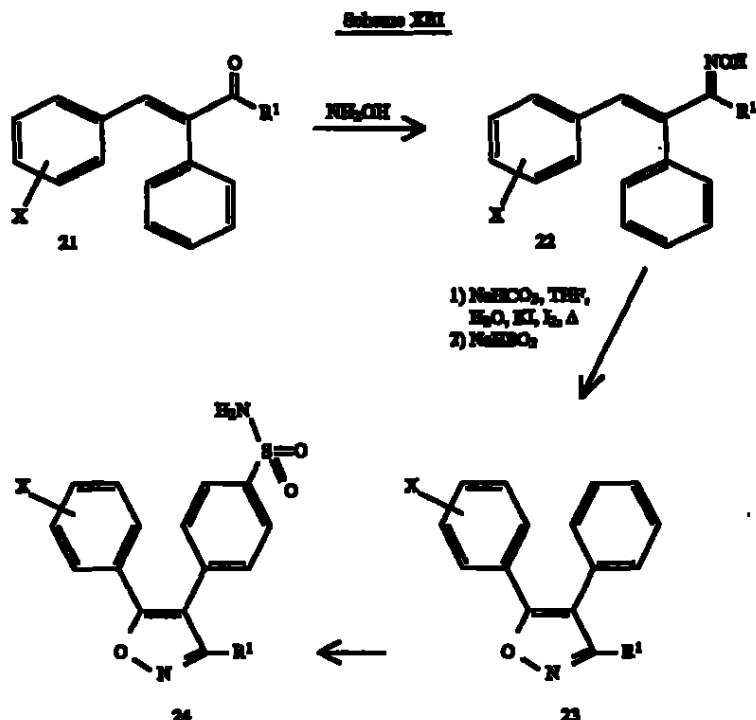
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wherein R^1 is selected from hydroxyl, lower alkyl, carbonyl, lower carboxyalkyl, lower aminocarbonylalkyl, lower alkoxy carbonylalkyl, lower aralkyl, lower alkoxyalkyl, lower aralkoxyalkyl, lower alkylthioalkyl, lower aralkylthioalkyl, lower alkylaminoalkyl, lower aryloxyalkyl, lower arylthioalkyl, lower haloalkyl, lower hydroxyalkyl, cycloalkyl, cycloalkylalkyl, and analkyl; wherein R^2 is selected from cycloalkyl, cycloalkenyl, aryl, and heteroaryl; wherein R^3 is optionally substituted at a substitutable position with one or more radicals independently selected from lower alkylsulfonyl, lower alkyl, cyano, carbonyl, lower alkoxy carbonyl, lower haloalkyl, hydroxyl, lower hydroxyalkyl, lower haloalkoxy, amino, lower alkylamino, lower arylamino, lower aminoalkyl, nitro, halo, lower alkoxy, aminosulfonyl, and lower alkylthio; and wherein R^4 is selected from lower alkyl, hydroxyl and amino; or a pharmaceutically-acceptable salt thereof.

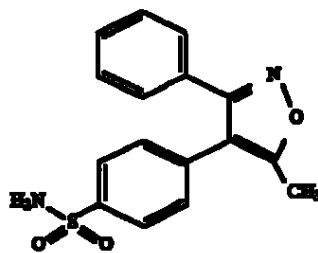
29 Claims, No Drawings

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Synthetic Scheme XIII shows the three step procedure used to prepare 4,5-diphenylisoxazole antiinflammatory agents 24 from 1,2-diphenylbutanones 21. In step one, the 1,2-diphenylbutanones 21 are converted to the corresponding oximes 22 by treatment with hydroxylamine in a manner similar to that shown in Scheme IV. In step two, the oxime 22 is converted to the 4,5-diphenylisoxazole 23 in two steps. The oxime 22 is reacted with potassium iodide and iodine in the presence of base, such as sodium bicarbonate and heated to form the halo intermediate. Sodium bisulfite is added to form the isoxazole 23. The isoxazole 23 is converted to the sulfonamide by any of the procedures shown in Schemes VIII, XI or XII.

The following examples contain detailed descriptions of the methods of preparation of compounds of Formulas I-IV. These detailed descriptions fall within the scope, and serve to exemplify, the above described General Synthetic Procedures which form part of the invention. These detailed descriptions are presented for illustrative purposes only and are not intended as a restriction on the scope of the invention. All parts are by weight and temperatures are in Degrees centigrade unless otherwise indicated. All compounds showed NMR spectra consistent with their assigned structures.

EXAMPLE 1



Valdecoxib

4-[5-Methyl-3-phenylisoxazol-4-yl]
benzenesulfonamide

Step 1. Preparation of desoxybenzoic keto-oxime.

Desoxybenzoic (20.0 g, 0.102 mol) was dissolved in toluene (200 mL). In a separate 500 mL round-bottom flask equipped with a magnetic stirring bar, hydroxylamine hydrochloride (9.21 g, 0.132 mol) and potassium hydroxide (7.43 g, 0.132 mol) were suspended in absolute ethanol (50 mL) and stirred vigorously at room temperature for thirty minutes. The desoxybenzoic solution was added in one portion, and the yellow suspension was held at reflux, using a Dean-Stark trap to remove generated water, under a nitrogen blanket for 16 hours. The suspension was cooled to room temperature and poured into water (200 mL). The

[54] SUBSTITUTED PYRAZOLYL
BENZENESULFONAMIDES FOR THE
TREATMENT OF INFLAMMATION

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[73] Assignee: G.D. Searle & Co., Skokie, Ill.

[21] Appl. No.: 608,113

[22] PCT Filed: Nov. 14, 1994

[86] PCT No.: PCT/US94/12720

§ 371 Date: Sep. 6, 1996

§ 102(e) Date: Sep. 6, 1996

[87] PCT Pub. No.: WO96/15316

PCT Pub. Date: Jun. 8, 1995

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 223,629, Apr. 6, 1994, Pat. No. 5,521,207, which is a continuation-in-part of Ser. No. 160,594, Nov. 30, 1993, Pat. No. 5,466,823.

[51] Int. Cl.⁶ A61K 31/415

[52] U.S. Cl. 514/483; 514/406; 514/407

[58] Field of Search 514/403, 406, 514/407

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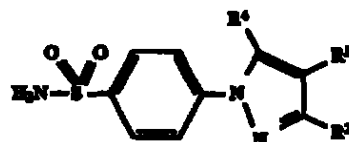
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Primary Examiner—Robert W. Rammner
Attorney, Agent, or Firm—Joseph W. Bullock

[57] ABSTRACT

A class of pyrazolyl benzenesulfonamide compounds is described for use in treating inflammation and inflammation-related disorders. Compounds of particular interest are defined by Formula II:



or a pharmaceutically-acceptable salt thereof.

18 Claims, No Drawings

celecoxib
deracoxib

D3

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dosage units are tablets or capsules. The active ingredient may also be administered by injection as a composition wherein, for example, saline, dextrose or water may be used as a suitable carrier.

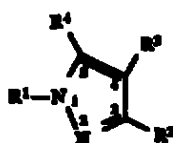
The amount of therapeutically active compound that is administered and the dosage regimen for treating a disease condition with the compounds and/or compositions of this invention depends on a variety of factors, including the age, weight, sex and medical condition of the subject, the severity of the disease, the route and frequency of administration, and the particular compound employed, and thus may vary widely. The pharmaceutical compositions may contain active ingredient in the range of about 0.1 to 2000 mg, preferably in the range of about 0.5 to 500 mg and most preferably between about 1 and 100 mg. A daily dose of about 0.01 to 100 mg/kg body weight, preferably between about 0.1 and about 50 mg/kg body weight and most preferably from about 1 to 20 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

For therapeutic purposes, the compounds of this invention are ordinarily combined with one or more adjuvants appropriate to the indicated route of administration. If administered per os, the compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of stearic acid, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain a controlled-release formulation as may be provided in a dispersion of active compound in hydroxypropylmethyl cellulose. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules having one or more of the carriers of diluents mentioned for use in the formulations for oral administration. The compounds may be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations.

What is claimed is:

1. A method of treating inflammation or an inflammation-associated disorder in a subject, said method comprising administering to the subject having or susceptible to such inflammation or inflammation-associated disorder, a therapeutically-effective amount of a compound of Formula I



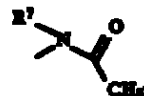
wherein R^1 is phenyl substituted at a substitutable position with one or more radicals selected from halo, C_1 - C_{10} -alkyl, and sulfamyl

wherein R^2 is selected from hydrido, C_1 - C_6 -haloalkyl, cyano, carboxy, C_1 - C_6 -alkoxycarbonyl, C_1 - C_6 -carboxyalkyl, C_1 - C_6 -alkoxy, C_1 - C_6 -alkylthio,

aminocarbonyl, aminocarbonyl- C_1 - C_6 -alkyl, C_1 - C_6 -N-alkylaminocarbonyl, N-arylamino carbonyl, C_1 - C_6 -N,N-dialkylaminocarbonyl, C_1 - C_6 -N-alkyl-N-arylaminocarbonyl, and C_1 - C_6 -hydroxyalkyl;

wherein R^3 is selected from hydrido, C_1 - C_{10} -alkyl, halo, cyano, C_1 - C_6 -alkoxy, C_1 - C_6 -hydroxyalkyl, C_1 - C_6 -alkylthio, and C_1 - C_6 -alkylsulfonyl;

wherein R^4 is selected from aryl- C_2 - C_6 -alkenyl, aryl, C_2 - C_7 -cycloalkyl, C_2 - C_7 -cycloalkenyl and five to ten membered heterocyclic; wherein R^4 is optionally substituted at a substitutable position with one or more radicals selected from halo, C_1 - C_6 -alkylthio, C_1 - C_6 -alkylsulfonyl, C_1 - C_{10} -alkyl, C_1 - C_6 -alkylsulfonyl, cyano, carboxyl, C_1 - C_6 -alkoxycarbonyl, aminocarbonyl, C_1 - C_6 -haloalkyl, hydroxyl, C_1 - C_6 -alkoxy, C_1 - C_6 -hydroxyalkyl, C_1 - C_6 -haloalkoxy, sulfamyl, C_1 - C_6 -N-alkylaminocarbonyl, amino, C_1 - C_6 -N-alkylamino, C_1 - C_6 -N,N-dialkylamino, five or six membered heterocyclic, nitro, and



and

wherein R^7 is hydrido;

wherein aryl wherever occurring means phenyl, naphthyl, tetrahydronaphthyl, indane, biphenyl,

provided R^2 and R^3 are not identical radicals selected from hydrido, carboxyl and ethoxycarbonyl; further provided that R^2 is not carboxyl or methyl when R^3 is hydrido and when R^4 is phenyl; further provided that R^4 is not triazolyl when R^2 is methyl; further provided that R^4 is not aralkenyl when R^2 is carboxyl, aminocarbonyl or ethoxycarbonyl; further provided that R^4 is not phenyl when R^2 is methyl and R^3 is carboxyl; further provided that R^4 is not 4-chlorophenyl when R^2 is methyl and R^3 is bromo; further provided that R^4 is not unsubstituted thienyl when R^2 is trifluoromethyl; and further provided that R^4 is aryl substituted with sulfamyl when R^2 is phenyl not substituted with sulfamyl; and further provided the compound is not 4-[5-(3-fluoro-4-methoxyphenyl)-3-(difluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide; or a pharmaceutically-acceptable salt thereof.

2. The method of claim 1 wherein R^1 is phenyl, substituted at a substitutable position with one or more radicals selected from fluoro, chloro, methyl, and sulfamyl;

wherein R^2 is selected from hydrido, fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, pentafluoroethyl, heptafluoropropyl, difluorochloromethyl, dichlorofluoromethyl, difluoroethyl, difluoropropyl, dichloroethyl, dichloropropyl, cyano, carboxyl, methoxycarbonyl, ethoxycarbonyl, isopropoxycarbonyl, tert-butoxycarbonyl, propoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl, pentoxycarbonyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, and hexamoyl, methoxy, ethoxy, methylthio, aminocarbonyl, aminocarbonylmethyl, N-methylaminocarbonyl, N-ethylaminocarbonyl, N-isopropylaminocarbonyl, N-propylaminocarbonyl, N-butylaminocarbonyl, N-isobutylaminocarbonyl, N-tert-butylaminocarbonyl, N-pentylaminocarbonyl, N-phenylaminocarbonyl,

4-[5-(4-(N-ethyl-N-methylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(3-fluoro-4-(N-methylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(3-chloro-4-(N-methylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(3-methyl-4-(N-methylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(4-(N,N-dimethylamino)-3-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(3-chloro-4-(N,N-dimethylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(4-(N,N-dimethylamino)-3-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(4-(N-ethyl-N-methylamino)-3-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(3-chloro-4-(N-ethyl-N-methylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(4-(N-ethyl-N-methylamino)-3-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(4-(N,N-diethylamino)-3-fluorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(4-(3-chloro-4-(N,N-diethylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(4-(N,N-diethylamino)-3-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
N-[4-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-3-fluorophenyl]-N-methylacetamide;
N-[4-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-3-chlorophenyl]-N-methylacetamide;
N-[4-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-3-methylphenyl]-N-methylacetamide;
N-[4-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-3-fluorophenyl]-N-methylurea;
N-[4-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-3-chlorophenyl]-N-methylurea;
N-[4-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-3-methylphenyl]-N-methylurea;
N-[4-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-3-fluorophenyl]-N-methylthiourea;
N-[4-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-3-chlorophenyl]-N-methylthiourea;
N-[4-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-3-methylphenyl]-N-methylthiourea;
4-[5-(3-(N,N-dimethylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(3-(N-ethyl-N-methylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(4-chloro-3-(N-methylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
4-[5-(4-methyl-3-(N-methylamino)phenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
N-[3-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]phenyl]-N-methylacetamide;
N-[3-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-4-fluorophenyl]-N-methylacetamide;
N-[3-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-4-methylphenyl]-N-methylurea;
N-[3-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-4-fluorophenyl]-N-methylthiourea;
4-[5-(2-(N-ethyl-N-methylamino)-4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide;
N-[2-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-4-methylphenyl]-N-methylurea;
N-[2-[1-[4-(aminosulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazol-5-yl]-4-fluorophenyl]-N-methylthiourea;
4-[5-(1H-indol-5-yl)-3-trifluoromethyl]-1H-pyrazol-1-yl]benzenesulfonamide;

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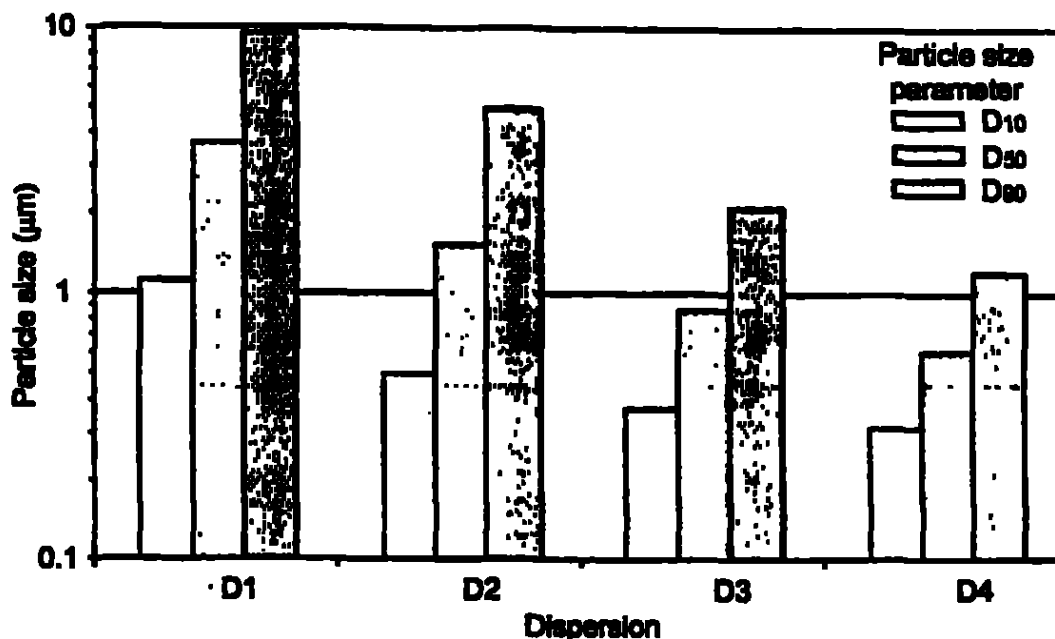
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(54) Title: CYCLOOXYGENASE-2 INHIBITOR COMPOSITIONS HAVING RAPID ONSET OF THERAPEUTIC EFFECT



(57) Abstract: Pharmaceutical compositions are provided comprising one or more orally deliverable dose units, each comprising a selective cyclooxygenase-2 inhibitory drug of low water solubility in a therapeutically effective amount, wherein the drug is present in the form of solid particles, about 25 % to 100 % by weight of which are smaller than 1 µm. The compositions are useful in treatment or prophylaxis of cyclooxygenase-2 mediated conditions and disorders and have particular advantages where rapid onset of therapeutic effect is desired.

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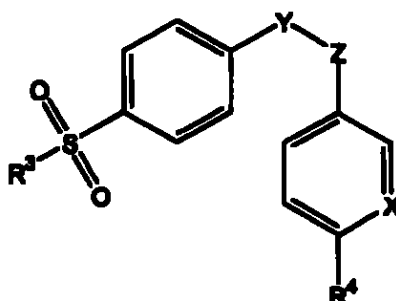
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448**WHAT IS CLAIMED IS:**

1. A pharmaceutical composition comprising one or more orally deliverable dose units, each comprising a selective cyclooxygenase-2 inhibitory drug of low water solubility in a therapeutically effective amount, wherein the drug is present in solid particles having a D_{90} particle size of about 0.01 μm to about 200 μm , a sufficient portion by weight of the particles being smaller than 1 μm to provide a substantially higher C_{max} and/or a substantially shorter T_{max} by comparison with an otherwise similar composition wherein substantially all of the particles are larger than 1 μm .
2. A pharmaceutical composition comprising one or more orally deliverable dose units, each comprising a selective cyclooxygenase-2 inhibitory drug of low water solubility in a therapeutically effective amount, wherein the drug is present in solid particles having a D_{90} particle size of about 0.01 μm to about 200 μm , and wherein about 25% to 100% by weight of the particles are smaller than 1 μm .
3. The composition of Claim 1 or Claim 2 wherein substantially all of the particles are smaller than 1 μm .
4. The composition of any of Claims 1 to 3 wherein the dose units are in the form of discrete solid articles.
5. The composition of Claim 4 wherein the solid articles are tablets or capsules.
6. The composition of any of Claims 1 to 3 that is in the form of a substantially homogeneous flowable mass from which single dose units are measurably removable.
7. The composition of Claim 6 wherein the substantially homogeneous flowable mass is a liquid suspension.
8. The composition of any of Claims 1 to 7 wherein the solid particles have a D_{25} particle size of about 450 nm to about 1000 nm.
9. The composition of any of Claims 1 to 7 wherein about 25% to 100% by weight of the solid particles have a particle size of about 450 nm to about 1000 nm.
10. The composition of any of Claims 1 to 7 wherein the solid particles have a

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weight average particle size of about 450 nm to about 1000 nm.

11. The composition of any of Claims 1 to 10 wherein the selective cyclooxygenase-2 inhibitory drug is a compound of formula



where R^3 is a methyl or amino group, R^4 is hydrogen or a C_{1-4} alkyl or alkoxy group, X is N or CR^5 where R^5 is hydrogen or halogen, and Y and Z are independently carbon or nitrogen atoms defining adjacent atoms of a five- to six-membered ring that is unsubstituted or substituted at one or more positions with oxo, halo, methyl or halomethyl groups.

12. The composition of Claim 11 wherein the five- to six-membered ring is selected from the group consisting of cyclopentenone, furanone, methylpyrazole, isoxazole and pyridine rings substituted at no more than one position.
13. The composition of any of Claims 1 to 10 wherein the selective cyclooxygenase-2 inhibitory drug is selected from the group consisting of celecoxib, deracoxib, valdecoxib, rofecoxib, 5-chloro-3-(4-methylsulfonyl)phenyl-2-(2-methyl-5-pyridinyl)pyridine, 2-(3,5-difluorophenyl)-3-[4-(methylsulfonyl)phenyl]-2-cyclopenten-1-one and (S)-6,8-dichloro-2-(trifluoromethyl)-2H-1-benzopyran-3-carboxylic acid.
14. The composition of Claim 13 wherein the selective cyclooxygenase-2 inhibitory drug is celecoxib.
15. The composition of Claim 14 comprising about 10 mg to about 1000 mg celecoxib in each dose unit.
16. A method of treating a medical condition or disorder in a subject where treatment with a cyclooxygenase-2 inhibitor is indicated, comprising orally administering one or more dose units of a composition of any of Claims 1 to 15 one to about six times a day.

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17. The method of Claim 16 wherein the medical condition or disorder is accompanied by acute pain.
18. A method of use of solid particles of a selective cyclooxygenase-2 inhibitory drug of low water solubility in manufacture of a medicament useful in treatment or prophylaxis of a COX-2 mediated condition or disorder, said solid particles having a D_{50} particle size of about 0.01 μm to about 200 μm , and wherein about 25% to 100% by weight of the solid particles are smaller than 1 μm .



UNIVERSIDAD NACIONAL DE COLOMBIA

Sede Bogotá

Facultad de Ciencias

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Respetado Doctor Barberi:

En relación al documento técnico elaborado por el Departamento de Farmacia y entregado a Ustedes en el mes de Octubre de 2003 respecto a "Importancia del tamaño de partícula de un material en el campo farmacéutico", nos permitimos hacer entrega de las conclusiones extraídas del mencionado documento.

DOCUMENTO TÉCNICO- CONCLUSIONES

Elaborado por: Luisa Fernanda Ponce D'León. Química Farmacéutica, Doctor en Química, Magíster en Fisicoquímica, Especialista en Buenas Prácticas de Manufactura, Estudios de Estabilidad y Bioequivalencia, Asesor temporero de la OPS en Buenas Prácticas de Manufactura, Profesora Asociada. Departamento de Farmacia. Universidad Nacional de Colombia.

Fecha de entrega del documento: 15 de Octubre de 2003

Conclusiones:

1. El tamaño y la forma de las partículas tienen influencia directa sobre la disolución, biodisponibilidad y eficacia de los fármacos, además de ser importantes para efectos de definir ciertos aspectos de su formulación final.
2. La granulometría es un procedimiento general que consiste en establecer el tamaño de partícula adecuado para elaborar productos farmacéuticos, en función de las necesidades que en cada caso se determinan en materia de disolución y biodisponibilidad, y de las conveniencias de empaque, costo, presentación o conservación del producto.
3. Desde tiempo muy antiguos se ha recurrido a la reducción del tamaño de materiales sólidos por pulverización, micronización, nanoparticulanización, etc.: entre otros métodos, con el objeto de acelerar su disolución. Dichas técnicas permiten aumentar la superficie específica del sólido, para lograr en un aumento en la dispersabilidad y velocidad de disolución, seguida de una mejor absorción y en consecuencia, por una mayor biodisponibilidad.

LA SOLUBILIDAD ACUOSA DEL FÁRMACO COMO FACTOR DETERMINANTE DE SU BIODISPONIBILIDAD

La solubilidad acuosa del fármaco desempeña un papel muy importante en el diseño y formulación de los medicamentos. El profesor Hemming G. Kristensen del Departamento de Farmacia de la Escuela Real de Farmacia de Copenhague afirma que los fármacos utilizados en medicamentos para uso personal deben tener una solubilidad en el agua superior a 10 mg/mL en el rango de pH comprendido entre 1 y 7, pues de lo contrario, se pueden presentar problemas en su absorción.

La solubilidad acuosa de un fármaco menor de 1 mg/mL presentará una velocidad de disolución limitada por cuanto la solubilidad y la velocidad de disolución están interrelacionadas.

Según el profesor Kristensen más del 40% de los nuevos fármacos aprobados en la Farmacopea Europea entre los años 1992 y 1995, presentan solubilidades menores a 1 mg/mL y el 32% tienen una solubilidad menor al 0.1 %.

El planteamiento del Profesor Kristensen coincide con las opiniones de emitidas por investigadores de laboratorios farmacéuticos norteamericanos como el Dr. Hauss de Eli Lilly.

Comenta el Dr. Hauss que cerca de la mitad de los nuevos fármacos obtenidos por Química Combinatoria tienen una muy reducida solubilidad en el agua.

Los químicos farmacéuticos de Investigación y Desarrollo de nuestra industria farmacéutica están de acuerdo en la afirmación que se plantea: más de un tercio de los fármacos listados en la USP 26 podrían catalogarse en la categoría de insolubles o muy poco solubles en el agua.

ALTERNATIVAS PARA CONTRARRESTAR EL PROBLEMA TECNOLÓGICO

Históricamente fué en la década de los años 60 que se planteó que cuando la solubilidad de un fármaco era inferior a 1 mg/mL, el tamaño de partícula (o área efectiva de superficie), representaba un factor importante a considerar para asegurar la biodisponibilidad del mismo.

Cabe anotar que con anterioridad a la década de los 60, J.G. Rheinold y colaboradores en 1945 observaron niveles plasmáticos más elevados con una suspensión de sulfadiazina micronizada (tamaño 1 a 3 μ) que aquella que se venía utilizando con macrocristales del fármaco.

Con la iniciación de las ciencias biofarmacéuticas en la década de los años 60 se establece la importancia que tiene la relación entre las propiedades fisicoquímicas de un fármaco en una forma de dosificación y la respuesta terapéutica observada después de su administración.

Si bien la micronización del fármaco solucionó parcialmente el problema de biodisponibilidad de fármaco de baja solubilidad, también causó problemas en formas unitarias de administración sólidas por fenómenos de aglomeración, problemas de carga estática con la consecuente segregación de las mezclas sólidas. Se trabajó también en sistemas de administración líquida utilizando alcohol y/o propilenglicol para solubilizar el fármaco, el cual precipitaría en partículas micronizadas a nivel gástrico, al diluirse con los fluidos biológicos.

Otro sistema utilizado para subdivisión de fármacos poco solubles en agua y que tiene vigencia, lo constituyen las llamadas dispersiones sólidas cuya clasificación corresponde a:

- Mezclas eutécticas simples
- Soluciones sólidas
- Soluciones y suspensiones vítreas
- Precipitaciones amorfas en un portador cristalino
- Formación de complejos
- Asociaciones entre algunas de las anteriores

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En la actualidad los métodos disponibles para incrementar la velocidad de disolución de fármacos de baja solubilidad en el agua corresponden a:

- Incremento del área superficial
- Formación de complejos con excipientes
- Solubilización utilizando tensioactivos
- Cambio de la forma cristalina por precipitación con polímeros hidrofílicos
- Formulación lipofílica
- Preparación de dispersiones sólidas
- Formación de sales de ácidos y bases débiles
- Utilización de profármacos
- Nanoestructuración del fármaco por tecnología criogénica
- Comición farmacológica - tensioactiva

Effect of the Physicochemical Properties of the Drug on Dissolution Rate

Factors that affect the dissolution rate of dosage forms can be classified under three main categories; first, factors relating to the physicochemical properties of the drug; second, factors relating to the dosage form; and third, factors relating to the dissolution apparatus and the test parameters. The physicochemical properties of the drug substance that affect its dissolution rate include solubility, particle size and crystalline state such as polymorphism, state of hydration, solvation and complexation.

Other physical properties such as density, viscosity and wettability contribute to the general dissolution problems of flocculation, flotation and agglomeration. Adsorption characteristics of the drug have also been found to have a significant effect on the dissolution of certain drugs.

Drug Solubility

Solubility of the drug substance plays a prime role in controlling its dissolution from the dosage form. According to the modified Noyes and Whitney equation

$$R = \frac{dc}{dt} = k \frac{DS}{sh} (c_s - c_t) = k_1 (c_s - c_t) \quad (4-1)$$

and, under sink condition

$$R = \frac{dc}{dt} = k_2 c_s \quad (4-2)$$

The above equations show that the aqueous solubility of the drug (c_s) is the major factor that determines its dissolution rate. According to Eq 4-2 a plot of R , the dissolution rate, vs c_s should yield a straight line which passes through the origin and has a slope equal to k_2 .

Hamlin, *et al.*¹ studied 45 compounds representing many different chemical classes

and a wide range of solubilities to demonstrate that the initial dissolution rates of these substances directly are proportional to their respective solubilities. Under their experimental conditions, the authors found that, $R = 2.24c_s$. Using this empirical relationship, it was suggested that it is possible to predict the approximate initial rates of dissolution of a broad range of compounds when their solubility values are known. Furthermore, the solubility data may be used as a rough predictor of the likelihood of any future problems with bioavailability. For example, it has been advocated² that a minimum aqueous solubility of 1% is required to avoid potential solubility-limited absorption problems. Parrott, *et al.*³ investigated the effect of solubility of benzoic acid on its dissolution rate from compressed tablets. Figure 4-1 shows that the dissolu-

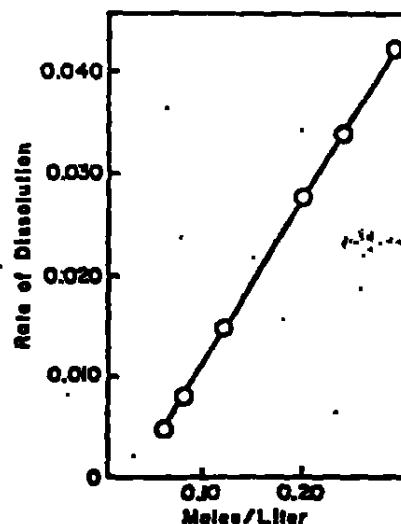


Fig 4-1. Influence of the solubility of benzoic acid on the dissolution rate in sodium sulfate solutions. [Reproduced from Parrott, *et al.*, *J Pharm Sci*, 44: 269 (1955).]

Dissolution, Bioavailability and Bioequivalence

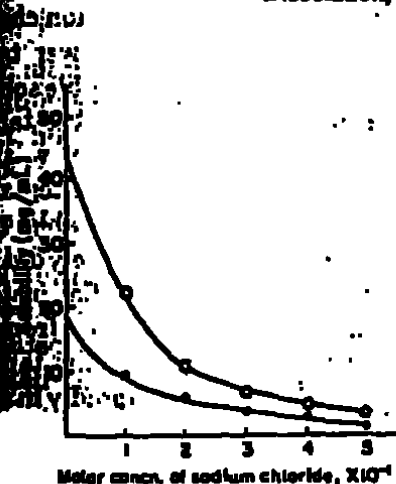


Fig 4-6a. Effect of sodium chloride concentration on the aqueous solubility of papaverine-HCl at 37°C. [Reproduced from Miyazaki, et al, *Chem Pharm Bull*, 29, 3414 (1981).]

Key: $\circ$ —, water; $\bullet$ —, 0.1N HCl.

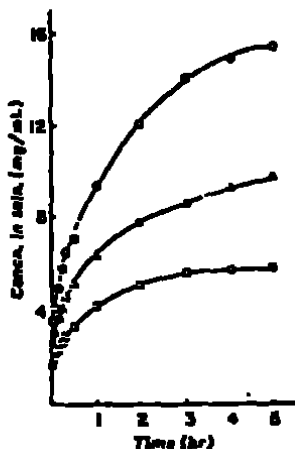


Fig 4-6b. Dissolution behavior of papaverine-HCl in 0.1N HCl at 37°C, in the presence and absence of sodium chloride. [Reproduced from Miyazaki, et al, *Chem Pharm Bull*, 29, 3414 (1981).]

Key: $\circ$ —, 0.1N HCl; $\triangle$ —, 0.9% NaCl in 0.1N HCl; $\square$ —, 0.9% NaCl in 0.1N HCl.

ained at pH 3.7. Similarly, the dissolution rates of the free bases of papaverine, trihexyphenidyl, and isoxsuprine were much higher than the hydrochloride salts at the pH range of the stomach (pH 1.0–1.4).

Miyazaki, et al,²⁶ confirmed the inhibitory action of the chloride ion on the solubility and dissolution rate of papaverine HCl not only *in vitro* (using a medium rich in chloride ion) but also *in vivo*. When the drug was administered to rats with 0.9% NaCl, it resulted in a decrease in the drug-

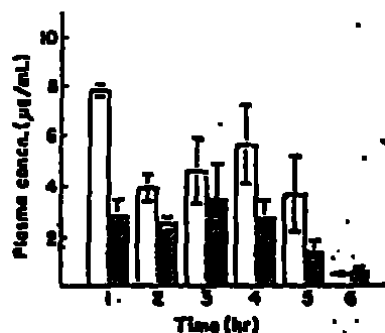


Fig 4-6c. Effect of chloride ion on the plasma drug levels following oral administration of papaverine-HCl suspensions to rats. [Reproduced from Miyazaki, et al, *Chem Pharm Bull*, 29, 3414 (1981).]

Key: $\square$, water; $\blacksquare$, 0.9% NaCl.

blood level from that obtained when the drug was administered alone. The authors interestingly, suggested that it may be possible to formulate sustained release preparations of papaverine HCl by combination with NaCl (Figs. 4-6a, 4-6b, and 4-6c). The common ion effect was also demonstrated²⁷ for other amine hydrochloride salts such as phenazopyridine HCl, cyproheptadine HCl, and bromhexine HCl. These findings are of extreme importance for the research pharmacist who has to decide which form of the active ingredient to use in formulating a new drug.

Concerns over slow dissolution and poor bioavailability traditionally have been limited to the sparingly soluble free acids. According to the above discussion, the concerns should be extended equally to the freely soluble salts as well. This also points to the importance of selecting the proper dissolution medium, if a meaningful *in vitro/in vivo* comparison is sought.

Particle Size

Equation 4-1 shows a direct relationship between the surface area of the drug and its dissolution rate. Since the surface area increases with decreasing particle size, higher dissolution rates may be achieved through reduction of the particle size. Under sink conditions, according to Eq 4-2, a plot of dissolution rate vs time should give a straight line with a slope of k . However,

Enhanced Drug Dissolution and Absorption

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The aqueous solubility of drug substances plays an important role in the formulation of drug dosage forms. For the oral route of administration it is well experienced that, unless the substance has an aqueous solubility above 10 mg/ml over the pH-range 1-7, then potential absorption problems may occur. A solubility less than 1 mg/ml is likely to give dissolution-rate limited absorption because solubility and dissolution rate are interrelated.

The proportion of drug substances showing aqueous solubilities below 1 mg/ml seems to increase steadily along with increasing specificity in the biological action of new chemical substances. A review of new monographs (1992-1995) in the European Pharmacopoeia by the author shows that more than 40 per cent of the drug substances have aqueous solubilities below 1 mg/ml, and that 32 per cent have an aqueous solubility below 0.1 mg/ml. The implementation of dissolution and absorption enhancing methods is, therefore, a major field in the formulation of drug dosage forms, in particular for the oral route of administration.

A wide range of principles and methods is available for the purpose of enhancing dissolution and/or dissolution rate of 'low-solubility' substances, for example:

- selection of salt form for weak acids and bases,
- reduction of particle size and, thereby, increased specific surface area,
- use of surfactants for increased wettability,
- complex formation with excipients, e.g. hydrophilic polymers or cyclodextrins,
- preparation of solid dispersions,
- change of crystal form by precipitation with hydrophilic polymers,
- lipophilic formulations, i.e. emulsions, microemulsions etc.,

Selection of an appropriate salt form - or in case of liquid preparations, adjustment of pH - is first choice for acidic and basic drug substances. The selection of a salt form is essentially a preformulation task because the choice has implications to the regulatory aspects of drug research and development. The survey of monographs in the European Pharmacopoeia shows that the main part of substances having aqueous solubilities above 10 mg/ml was hydrochlorides, sulphates, maleates, citrates etc of basic drugs or for acidic drugs potassium, sodium, calcium or other salts. Once the proper drug substance has been selected, preformulation studies are needed to characterise the physical and physico-chemical properties of the substance, e.g. pKa, pH-solubility profile, intrinsic solubility and dissolution rate, polymorphisms, hygroscopicity, log P etc. Although these characteristics may give some idea about potential absorption and bioavailability problems, there is, at present, insufficient knowledge about the systematics on how to formulate a given substance. It is difficult to predict the factors limiting the dissolution and absorption rates. It is believed that present industrial practise is the trial and error method based upon laboratory experiments and animal testing.

Marketed formulations of very slightly soluble substances, for example steroids, show that particle size reduction and, hence, increased specific surface area are important means to achieve a satisfactory dissolution rate. In principle, the lower the aqueous solubility, the lower particle size is required. For the practically insoluble substances (< 0.1 mg/ml), the required particle size is so low that it may be technically difficult to prepare the desired size range. Significant progress has been made in recent years in the field of 'nano-particle' technology. On the other hand, physical instability of the size distribution is introduced when the size range is reduced to micrometer and sub-micrometer scale.

Physical instability is a major problem also with other dissolution enhancing methods where the state of the drug substance is made 'high energetic', i.e. solid dispersions and coprecipitates with hydrophilic polymers where the drug substance typically is present in an amorphous state. Despite the great interest these systems had 1-2 decades ago, few products based on such means have

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

2020
Bogotá, D.C., Septiembre 5 de 2008

Abogado
EMILIO FERRERO

ASUNTO	Radicación No.	01 087 299
	Trámite	002
	Evento	001
	Actuación	430
	Oficio No.	10451
	Folios	0

Como resultado del examen de patentabilidad de la solicitud de patente de invención, realizado según lo establece el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se notifica el siguiente concepto técnico:

Título de la solicitud: "Composiciones Inhibidoras de Ciclooxigenasa-2 que tiene rápido acceso de efecto terapéutico".

1. Documentos estudiados

Para el presente estudio se tuvieron en cuenta el capítulo descriptivo comprendido en los folios 4 - 117 y el capítulo reivindicatorio de los folios 118 - 123 de la solicitud.

2. Objeto de la Invención

El objeto de la presente solicitud de patente de invención consiste en:

Las reivindicaciones 1 - 7 se refieren a composiciones (tabletas, cápsulas, suspensión) que contienen inhibidores de COX-2 con tamaño de partícula D₉₀ de 0.01 - 200 micras.

Las reivindicaciones 8 - 10 se refieren a composiciones de las reivindicaciones 1 -7 donde el principio activo sólido tiene un tamaño de partícula D₂₅ de 250 - 1000 nm.

Las reivindicaciones 11 - 15 se refieren a composiciones de las reivindicaciones 1 - 10 que contienen compuestos tales como celecoxib, deracoxib, valdecoxib, rofecoxib y otros.

Las reivindicaciones 16 - 17 se refieren a un método de tratamiento que consiste en administrar una composición.

La reivindicación 18 se refiere al método de uso de una droga en la fabricación de un medicamento.

El problema planteado en esta solicitud es la necesidad de composiciones para tratamiento y prevención de desórdenes caracterizados por inflamación, dolor y/o fiebre, artritis.

Para resolver este problema técnico la solicitud en estudio proporciona composiciones farmacéuticas que contienen compuestos inhibidores de COX-2 con tamaño de partícula D_{50} de 0.01 – 200 micras ó D_{25} de 250 – 1000 nm, tales como celecoxib, deracoxib, valdecoxib, rofecoxib.

El objeto de la invención definido anteriormente se clasificó de acuerdo con la IPC: A 61 K 31/42; A 61 K 9/20; A61K31/415; A61K9/50

No se reivindica prioridad de una Solicitud de Patente anterior.

3. Excepciones a la Patentabilidad

El artículo 20 de la Decisión 486 establece: *"No serán patentables: d) los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales"*

Las reivindicaciones 16 – 17 se refieren a un método de tratamiento que consiste en administrar una composición.

Se observa que el método de tratamiento de las reivindicaciones 16 - 17 que comprende administrar una composición, no es patentable de acuerdo con la norma citada, que especifica que *los métodos terapéuticos para el tratamiento humano o animal no son patentables*.

En conclusión, el método de tratamiento de las reivindicaciones 16 - 17, que comprende administrar una composición, no es patentable de acuerdo con el Art 20, literal d), de la decisión 486.

4. Usos

El artículo 21 de la Decisión 486 de la Comisión de la Comunidad Andina establece que: *"Los productos o procedimientos ya patentados, comprendidos en el estado de la técnica, de conformidad con el artículo 16 de la presente Decisión, no serán objeto de nueva patente, por el simple hecho de atribuirse un uso distinto al originalmente comprendido por la patente inicial"*.

El artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina establece que *"Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial"*.

El Artículo 19 de la Decisión 486 de la Comisión de la Comunidad Andina establece que *"Se considerará que una invención es susceptible de aplicación industrial, cuando su objeto pueda ser producido o utilizado en cualquier tipo de industria, entendiéndose por industria la referida a cualquier actividad productiva, incluidos los servicios."*

La reivindicación 18 se refiere al método de uso de una droga en la fabricación de un medicamento.

Se aclara que, de acuerdo con el Art 14, no se puede otorgar patente a usos porque estos no son en sí, un producto ni un procedimiento tecnológico.

De otro lado, de acuerdo con el Art 19, se considera que los usos no tienen aplicación industrial porque no se refiere a un tipo de producción ó utilización en ningún tipo de Industria.

Además los usos de los compuestos Inhibidores de COX-2 con tamaño de partícula D_{90} de 0.01 – 200 micras ó D_{25} de 250 – 1000 nm, tales como celecoxib, deracoxib, valdecoxib, rofecoxib no pueden ser objeto de nueva patente, aunque el solicitante los considere distintos a los originalmente comprendidos por la patente inicial, porque estos se conocían originalmente en las Pat US 5 474 995, 5 633 272 y 5 760 068 y WO 01 41760.

5. Con respecto a los Art. 28 y 30 de la D 486

El artículo 28 de la Decisión 486 establece: *"La descripción deberá divulgar la invención de manera suficientemente clara y completa para su comprensión y para que una persona capacitada en la materia técnica correspondiente pueda ejecutarla. La descripción de la invención indicará el nombre de la invención e incluirá la siguiente información:*

- a) el sector tecnológico al que se refiere o al cual se aplica la invención;*
- b) la tecnología anterior conocida por el solicitante que fuese útil para la comprensión y el examen de la invención, y las referencias a los documentos y publicaciones anteriores relativas a dicha tecnología;*
- c) una descripción de la invención en términos que permitan la comprensión del problema técnico y de la solución aportada por la invención, exponiendo las diferencias y eventuales ventajas con respecto a la tecnología anterior;*
- d) una reseña sobre los dibujos, cuando los hubiera;*
- e) una descripción de la mejor manera conocida por el solicitante para ejecutar o llevar a la práctica la invención, utilizando ejemplos y referencias a los dibujos, de ser éstos pertinentes; y,*
- f) una indicación de la manera en que la invención satisface la condición de ser susceptible de aplicación industrial, si ello no fuese evidente de la descripción o de la naturaleza de la invención.*

El artículo 30 de la Decisión 486 establece: *"Las reivindicaciones definirán la materia que se desea proteger mediante la patente. Deben ser claras y concisas y estar enteramente sustentadas por la descripción.*

Las reivindicaciones podrán ser independientes o dependientes. Una reivindicación será independiente cuando defina la materia que se desea proteger sin referencia a otra reivindicación anterior. Una reivindicación será dependiente cuando defina la materia que se desea proteger refiriéndose a una reivindicación anterior. Una reivindicación que se refiera a dos o más reivindicaciones anteriores se considerará una reivindicación dependiente múltiple".

La descripción divulga la invención de manera suficientemente clara y completa para su comprensión, tal como lo establece el Art 28 de la decisión 486 de la Comisión Andina. Menciona el sector tecnológico al que pertenece, los antecedentes de la técnica, la divulgación del problema técnico, la solución propuesta y la ventaja frente a la tecnología anterior.

Pero en cuanto a las reivindicaciones 1 - 7 se observa que la expresión "porción suficiente" no es una característica definida ni clara de la composición.

El mencionar que puede suministrar una C_{max} ó una T_{max} mayor ó menor que otras composiciones, no define la composición ni la caracteriza. Se recuerda que el resultado a alcanzar no es una característica técnica de la invención. Las

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reivindicaciones deben definir la invención por sus características técnicas esenciales. La invención no queda definida por el resultado a alcanzar (tal como C_{max} ó una T_{max}), puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Debe hacerse la corrección necesaria.

Las unidades de las reivindicaciones 1 - 7 (micras) son diferentes de las unidades de las reivindicaciones 8 - 10 (nm). Se debe mencionar el mismo tipo de unidades. Debe hacerse la corrección necesaria.

6. Determinación del Estado de la Técnica

El artículo 16 de la decisión 486 de la comisión de la Comunidad Andina que establece que *"El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida."*

Solo para el efecto de la determinación de la novedad, también se considerará dentro del estado de la técnica, el contenido de una solicitud de patente en trámite ante la oficina nacional competente, cuya fecha de presentación o de prioridad fuese anterior a la fecha de presentación o de prioridad de la solicitud de patente que se estuviese examinando, siempre que dicho contenido esté incluido en la solicitud de fecha anterior cuando ella se publique o hubiese transcurrido el plazo previsto en el artículo 40".

Considerando que la fecha de presentación de la solicitud de patente es octubre 10 de 2001, se consultaron las bases de datos y los archivos con que cuenta la entidad con fecha de publicación anterior y se encontraron los siguientes documentos que anticiplan el objeto de esta solicitud:

No.	Documento	Título	Fecha de Publicación
D1	US 5 474 995	Phenyl heterocycles as cox-2 inhibitors	Dic 12, 1995
D2	US 5 833 272	Substituted isoxazoles for the treatment of inflammation	May 27, 1997
D3	US 5 760 068	Substituted pyrazolyl benzenesulfonamides for the treatment of inflammation	Jun 2, 1998
D4	WO 01 41760	Cyclooxygenase-2 Inhibitor Compositions Having Rapid Onset Of Therapeutic Effect	Jun 14, 2001
D5	Estudio Facultad Farmacia Universidad Nacional de Colombia	Concepto Técnico sobre: Tamaño de Partícula de Fármacos y su relación con la Biodisponibilidad y la Bioequivalencia de los Medicamentos	-
D6	Oposición 1 (folios 180 - 190)	PROCAPS S. A.	Abril 28, 2004
D7	Oposición 2 (folios 329 - 337)	Tecnoquímicas S. A.	Abril 28, 2004

7. Oposición 1

El opositor 1 hace las siguientes observaciones:

- ◆ Dice el opositor que previo a esta solicitud el estado de la técnica revelaba los documentos WO 01 41760 y WO 00 32189. Que WO 01 41760 revela con anterioridad composiciones farmacéuticas que contienen compuestos inhibidores de COX-2 con tamaño de partícula D_{90} de 0.01 - 200 micras ó D_{25} de 250 - 1000 nm, tales como celecoxib, deracoxib, valdecoxib, rofecoxib; que son idénticas a las que se reivindican ahora en esta solicitud, de manera que este documento afecta el requisito de novedad que

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menciona el Art 16 de la D 486.

- ♦ Que WO 00 32189 revela con anterioridad composiciones farmacéuticas (tabletas y suspensión) que contienen compuestos inhibidores de COX-2 tal como celecoxib con tamaño de partícula D_{50} de menos de 1 micra, y se aclara que la disminución de tamaño de partícula, aumenta la biodisponibilidad.

8. Oposición 2

El opositor 2 hace las siguientes observaciones:

- ♦ Dice que esta solicitud es idéntica a la Solicitud Colombiana No. 00 093 233 presentada el 6 de diciembre de 2000, la cual reivindicaba prioridad de la Solicitud de Patente de los Estados Unidos US 60 169 856 de 8 de diciembre de 1999. Que menciona los mismos principios activos, el mismo tipo de composiciones y los mismos tamaños de partícula. De manera que el objeto de la solicitud en estudio no es nuevo.
- ♦ Que la familia de principios activos y sus características de baja solubilidad eran conocidos en las Patentes de Estados Unidos US 5 474 995, US 5 633 272 . US 5 760 068. Y que eran conocidos y habituales en la industria los procesos de modificación de tamaño de partícula de los compuestos de baja solubilidad, con el fin de aumentar su velocidad de disolución. De manera que para una persona medianamente versada en la materia técnica era obvia la aplicación de procesos de modificación de tamaño de partícula para lograr una formulación como la de esta solicitud. De manera que el objeto de la solicitud en estudio no tiene nivel inventivo.
- ♦ Que el Expediente 98 005 805 se negó considerando que el disminuir el tamaño de partícula de un compuesto para variar su perfil de disolución y por lo tanto su biodisponibilidad, no constituye un avance del estado de la técnica porque cualquier persona versada en la técnica sabe que la disminución de tamaño de partícula aumenta el área superficial y consecuentemente la velocidad de disolución y por lo tanto la biodisponibilidad; como se explica en la "Farmacia de Remington". De manera que el objeto de la solicitud en estudio no tiene nivel inventivo.

9. Argumentos del Solicitante

El solicitante afirma en los folios 363 – 376 que existen estudios según los cuales no siempre el disminuir el tamaño de partícula aumenta la biodisponibilidad, porque esta está afectada, además de la velocidad de disolución, por la absorción, extracción hepática, fracción libre en plasma y peso molecular. P. ej.:

- ♦ En la primera referencia se observa que la carbamazepina 1 que tiene un tamaño de partícula más homogéneo tiene menor biodisponibilidad que carbamazepina 2 que tiene un tamaño de partícula más heterogéneo (partículas tienen diámetros muy diferentes). Agrega que se esperaba que la carbamazepina 1 (más homogénea) se disolviera y absorbiera más rápida y completamente que la carbamazepina 2 (más heterogénea). Pero en la práctica, ellos encontraron un tiempo de resorción mayor para la carbamazepina 2 (más heterogénea) y por lo tanto una mayor biodisponibilidad. Concluyen, entonces, que existen otras propiedades desconocidas de los cristales de carbamazepina que afectan la biodisponibilidad.
- ♦ En la segunda referencia se observó que la composición que tiene oxfendazol con tamaño de partícula 12 (micras) tiene menor

biodisponibilidad que las que tienen 1.65 y 3.2

- ♦ En la tercera referencia se observó que diltiazem existe en dos formas cristalinas diferentes: el cristal I (prismas) que tiene tamaño de partícula 400 – 500 micras y el cristal II (placas) que tiene tamaño de partícula 10 – 100. Se estudiaron las características de absorción de estas dos formas cristalinas y del polvo obtenido a partir de la forma cristalina I que tiene tamaño de partícula de 12 micras.

10. Respuesta a los Opositores

Se aclara que los Opositores tienen razón en todos los argumentos analizados. Así es que, según tales observaciones, el objeto de esta solicitud no tiene novedad y no tiene nivel inventivo.

11. Respuesta al Solicitante

Se aclara que:

- ♦ En la primera referencia se observa que la carbamazepina 1 que tiene un tamaño de partícula más homogéneo tiene menor biodisponibilidad que carbamazepina 2 que tiene un tamaño de partícula más heterogéneo (partículas tienen diámetros muy diferentes). Agrega que se esperaría que la carbamazepina 1 (más homogénea) se disolviera y absorbiera más rápida y completamente que la carbamazepina 2 (más heterogénea). Pero en la práctica, ellos encontraron un tiempo de resorción mayor para la carbamazepina 2 (más heterogénea) y por lo tanto una mayor biodisponibilidad. Concluyen, entonces, que existen otras propiedades desconocidas de los cristales de carbamazepina que afectan la biodisponibilidad. Es decir que lo que afectó en este estudio la biodisponibilidad, no fue propiamente el tamaño de partícula en sí, sino la distribución del tamaño de partícula, es decir que contiene partículas de tamaños diversos. Así que este estudio no disuadiría a una persona conocedora de lo que sucede en la mayor parte de los casos (es decir ver aumentada la velocidad de disolución y por lo tanto la velocidad de absorción y la biodisponibilidad como consecuencia de la disminución en tamaño de partícula) de realizar la disminución en tamaño de partícula para lograr un aumento en la velocidad de disolución y por lo tanto en la velocidad de absorción y la biodisponibilidad.
- ♦ En la segunda referencia se observó que la composición que tiene oxfendazol con tamaño de partícula 12 (micras) tiene menor biodisponibilidad que las que tienen 1.65 y 3.2. se aclara al solicitante que este estudio demuestra lo que es obvio, y esto es, que disminuyendo el tamaño de partícula, que originalmente era de 12 a 1.65 y 3.2, se logra un aumento en la velocidad de disolución y por lo tanto en la velocidad de absorción y la biodisponibilidad.
- ♦ En la tercera referencia se observó que diltiazem existe en dos formas cristalinas diferentes: el cristal I (prismas) que tiene tamaño de partícula 400 – 500 micras y el cristal II (placas) que tiene tamaño de partícula 10 – 100. Se estudiaron las características de absorción de estas dos formas cristalinas y del polvo obtenido a partir de la forma cristalina I que tiene tamaño de partícula de 12 micras. El objeto del estudio era comparar la biodisponibilidad entre las dos formas cristalinas y el polvo de diltiazem. Se encontró que después de la administración oral en solución acuosa, la biodisponibilidad del diltiazem es poco afectada por influencia de sus formas cristalinas. Y se concluye diciendo que la biodisponibilidad de diltiazem está afectada por otros factores como: la

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velocidad de disolución, por la absorción, extracción hepática, fracción libre en plasma y peso molecular. Así que el estudio en mención no compara la biodisponibilidad de varios polvos que tienen diverso tamaño de partícula, sino la influencia de sus formas cristalinas sobre la biodisponibilidad. De manera que este documento, contrario a lo que afirma el solicitante, no apartaría a un técnico de la solución propuesta: disminución de tamaño de partícula para mejorar la biodisponibilidad, que es el procedimiento al que normalmente recurre una persona conocedora de la materia.

12. Evaluación de los requisitos de Patentabilidad

El artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina establece que *"Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial"*.

En cumplimiento del artículo 14 de la Decisión 486, se procedió a evaluar el cumplimiento de los requisitos de novedad, nivel inventivo y aplicación industrial, con el fin de establecer la patentabilidad de la invención reivindicada en la solicitud en estudio.

Evaluación de la Novedad

El Art. 16 de la Decisión 486 de la Comisión de la Comunidad Andina establece: *"Una invención se considerará nueva cuando no está comprendida en el estado de la técnica. El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente, o, en su caso, de la prioridad reconocida."*

Solo para el efecto de la determinación de la novedad, también se considerará dentro del estado de la técnica, el contenido de una solicitud de patente en trámite ante la oficina nacional competente, cuya fecha de presentación o de prioridad fuese anterior a la fecha de presentación o de prioridad de la solicitud de patente que se estuviese examinando, siempre que dicho contenido esté incluido en la solicitud de fecha anterior cuando ella se publique o hubiese transcurrido el plazo previsto en el Art 40".

Las reivindicaciones 1 – 7 se refieren a composiciones (tabletas, cápsulas, suspensión) que contienen inhibidores de COX-2 con tamaño de partícula D_{90} de 0.01 – 200 micras.

Las reivindicaciones 8 – 10 se refieren a composiciones de las reivindicaciones 1 -7 donde el principio activo sólido tiene un tamaño de partícula D_{25} de 250 – 1000 nm.

Las reivindicaciones 11 – 15 se refieren a composiciones de las reivindicaciones 1 – 10 que contienen compuestos tales como celecoxib, deracoxib, valdecoxib, rofecoxib y otros.

Las reivindicaciones 16 – 17 se refieren a un método de tratamiento que consiste en administrar una composición.

La reivindicación 18 se refiere al método de uso de una droga en la fabricación de un medicamento.

D4 enseña: composiciones (tabletas, cápsulas, suspensión) que contienen inhibidores de COX-2 con tamaño de partícula D_{90} de 0.01 – 200 micras; composiciones donde el principio activo sólido tiene un tamaño de partícula D_{25}

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de 250 – 1000 nm; composiciones que contienen compuestos tales como celecoxib, deracoxib, valdecoxib, rofecoxib y otros; un método de tratamiento que consiste en administrar una composición; un método de uso de una droga en la fabricación de un medicamento.

Se observa que todos los elementos reivindicados en esta solicitud, es decir: *composiciones (tabletas, cápsulas, suspensión) que contienen inhibidores de COX-2 con tamaño de partícula D_{90} de 0.01 – 200 micras; composiciones donde el principio activo sólido tiene un tamaño de partícula D_{25} de 250 – 1000 nm; composiciones que contienen compuestos tales como celecoxib, deracoxib, valdecoxib, rofecoxib y otros; un método de tratamiento que consiste en administrar una composición; un método de uso de una droga en la fabricación de un medicamento;* ya habían sido revelados previamente en el estado de la técnica. De manera que el objeto de esta solicitud no es nuevo.

Evaluación del Nivel Inventivo

El artículo 18 de la Decisión 486 de la Comisión de la Comunidad Andina establece que *Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica.*

Las reivindicaciones 1 – 7 se refieren a composiciones (tabletas, cápsulas, suspensión) que contienen inhibidores de COX-2 con tamaño de partícula D_{90} de 0.01 – 200 micras.

Las reivindicaciones 8 – 10 se refieren a composiciones de las reivindicaciones 1 -7 donde el principio activo sólido tiene un tamaño de partícula D_{25} de 250 – 1000 nm.

Las reivindicaciones 11 – 15 se refieren a composiciones de las reivindicaciones 1 - 10 que contienen compuestos tales como celecoxib, deracoxib, valdecoxib, rofecoxib.

D1 enseña composiciones que contienen inhibidores de COX-2 fenil heterociclos tales como rofecoxib.

D2 enseña composiciones que contienen inhibidores de COX-2 isoxazoles sustituidos tales como valdecoxib.

D3 enseña composiciones que contienen inhibidores de COX-2 pirazolil bencensulfonamidas sustituidas tales como celecoxib, deracoxib.

D4 enseña *composiciones (tabletas, cápsulas, suspensión) que contienen inhibidores de COX-2 con tamaño de partícula D_{90} de 0.01 – 200 micras; composiciones donde el principio activo sólido tiene un tamaño de partícula D_{25} de 250 – 1000 nm; composiciones que contienen compuestos tales como celecoxib, deracoxib, valdecoxib, rofecoxib y otros; un método de tratamiento que consiste en administrar una composición; un método de uso de una droga en la fabricación de un medicamento.*

D5 enseña que la granulometría es un procedimiento general que consiste en definir el tamaño de partícula adecuado para elaborar productos farmacéuticos, en función de las necesidades de disolución y disponibilidad de cada caso y de las conveniencias de empaque, costo, presentación ó conservación del producto; agrega que modificar una propiedad física de un

sólido, en especial su tamaño de partícula con el fin de variar su perfil de disolución y determinar la variación de la biodisponibilidad, es una técnica ampliamente conocida en la industria farmacéutica. Y que los eventuales problemas de disolución ó biodisponibilidad de sustancias farmacéuticas pueden ser solucionados mediante la disminución de tamaño de partícula, por métodos, procesos y equipos de uso común en la industria farmacéutica; y también es común acudir al empleo de auxiliares de formulación que mejoren o faciliten la disolución.

Se observa que:

- (a) debido a que los compuestos inhibidores de COX-2 tales como celecoxib, deracoxib, valdecoxib, rofecoxib; eran conocidos en las anterioridades, de la misma manera que la característica de los principios activos de tener un tamaño de partícula D_{90} de 0.01 – 200 micras ó D_{25} de 250 – 1000 nm; una persona de habilidades ordinarias en el arte los adaptará fácilmente a la preparación de las composiciones reivindicadas en esta solicitud;
- (b) las reivindicaciones de esta solicitud mencionan tamaño de partícula D_{90} de 0.01 – 200 micras ó D_{25} de 250 – 1000 nm como una característica de los compuestos inhibidores de COX-2 tales como celecoxib, deracoxib, valdecoxib, rofecoxib; pero se observa que esta no les concede un efecto diferente o especial a los compuestos, ni a las composiciones, que pueda considerarse como un aporte a la técnica, así que son obvios para una persona versada en la materia;
- (c) el problema planteado en esta solicitud que era la necesidad de composiciones para tratamiento y prevención de desórdenes caracterizados por inflamación, dolor y/o fiebre, artritis se resuelve proporcionando composiciones farmacéuticas que contienen compuestos inhibidores de COX-2 tales como celecoxib, deracoxib, valdecoxib, rofecoxib, con tamaño de partícula D_{90} de 0.01 – 200 micras ó D_{25} de 250 – 1000 nm, es decir de la misma manera como se resolvía en las anterioridades;
- (d) el resultado obtenido: es decir composiciones farmacéuticas que contienen compuestos inhibidores de COX-2 tales como celecoxib, deracoxib, valdecoxib, rofecoxib, con tamaño de partícula D_{90} de 0.01 – 200 micras ó D_{25} de 250 – 1000 nm, no es inesperado.
- (e) no parece haberse superado un prejuicio técnico anterior, con las composiciones farmacéuticas que contienen compuestos inhibidores de COX-2 tales como celecoxib, deracoxib, valdecoxib, rofecoxib, con tamaño de partícula D_{90} de 0.01 – 200 micras ó D_{25} de 250 – 1000 nm propuestas de manera que el objeto reivindicado no tiene nivel inventivo.

13. Conclusión

En conclusión, los requisitos de patentabilidad de la presente solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US 5 474 985	1 – 15	Nivel Inventivo
D2	US 5 633 272	1 – 15	Nivel Inventivo
D3	US 5 760 068	1 – 15	Nivel Inventivo
D4	WO 01 41780	1 - 18	Novedad
D5	Estudio Facultad Farmacia Universidad Nacional de Colombia	1 – 15	Nivel Inventivo
D6	Oposición 1	1 – 15	Nivel Inventivo

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D7	Oposición 2	1 - 15	Nivel inventivo
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En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de la notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

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



ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

El anterior requerimiento fue notificado por fijación en lista, de fecha
24 SET. 2006

CONCEPTO TÉCNICO No. 1395	EXAMINADOR TÉCNICO Código 202007
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