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Referencia : Expediente No. 00.093.233

Asunto : Solicitud de patente de invención para:  
COMPOSICIONES INHIBIDORAS DE  
CICLOOXIGENASA-2 QUE TIENE RÁPIDO ACCESO  
DE EFECTO TERAPÉUTICO

Solicitante : PHARMACIA CORPORATION

Opositores : TECNOQUÍMICAS S.A. y LABORATORIO FRANCO  
COLOMBIANO LAFRANCOL 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 las sociedades TECNOQUÍMICAS S.A. y LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A., opositores en el asunto de la referencia.

## II. CONTESTACIÓN DENTRO DEL TÉRMINO LEGAL

El oficio No. 08389, 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 nueve (9) de octubre de dos mil tres (2003). El término previsto en el artículo 43 de la Decisión 486 de la Comisión de la Comunidad Andina vencía el nueve (9) de enero de dos mil cuatro (2004).

Mediante escrito radicado ante ese Despacho el ocho (8) de enero de dos mil cuatro (2004) solicité un plazo adicional de sesenta (60) días, para presentar las argumentaciones de mi representada, por lo que el nuevo término vence el seis (6) de abril de 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 las sociedades opositoras 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.** y **LABORATORIO FRANCO COLOMBIANO LAFRANCOL 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 LOS OPOSITORES**

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.

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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.

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, las sociedades TECNOQUÍMICAS S.A. y LABORATORIO FRANCO COLOMBIANO LAFRANCOL S.A. opositoras en el asunto de la referencia, presentan las razones que justifican su interés legítimo para ser consideradas por su Despacho como afectadas directamente en su interés por la solicitud de patente de mi representada. En el presente caso, las opositoras no han 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. y LABORATORIO FRANCO COLOMBIANO  
LAFRANCOL 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 las opositoras para concluir que no son 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 hacen las opositoras 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 prosperen 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.
- 5.4. En las solicitudes internacionales WO00/32189 y WO00/53149 se designan los mismos rangos de dosis de Celecoxib, así como los tamaños de partícula preferidos en la solicitud de la referencia, por lo que ésta carece de novedad y nivel inventivo.

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 las sociedades opositoras, 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 las opositoras 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** presentan las opositoras:

**6.1. Ausencia de novedad y nivel inventivo:**

Los opositores alegan que reducir el tamaño de partícula de un medicamento para lograr una mejor biodisponibilidad no constituye nivel inventivo. El argumento de los opositores 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, column 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:

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

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.)*

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

- 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:

*"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 ( $C_{pmax}$ ) y el tiempo necesario para alcanzar su pico ( $t_{max}$ ) 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 resaltado es nuestro).

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. Andrýsek 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 resaltado 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 T<sub>max</sub>, C<sub>max</sub> 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 submicrómetro. 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 Los opositores también citan dos documentos que afectan la patentabilidad de la invención reivindicada: WO 00/53149 y WO 00/32189:

WO 00/53149: La revelación de WO 00/53149 no es relevante para la patentabilidad de la invención reivindicada. En Colombia no se presentó un equivalente a WO 00/53149, por lo que la fecha efectiva de WO 00/53149 como referencia es su fecha de publicación, 14 de septiembre de 2000. Esta fecha es más de 9 meses posterior a la fecha de la prioridad de la presente solicitud, que es 9 de diciembre de 1999.

Ahora bien, si la revelación de WO 00/53149, se adujera como estado previo de la técnica, no convierte la invención reivindicada en una invención carente de nivel inventivo. WO 00/53149 revela composiciones alimenticias que contienen inhibidores de ciclooxigenasa-2 (v.g., alimentos para perros y gatos). WO 00/53149 contiene la siguiente enseñanza sobre la molienda del celecoxib en partículas:

*"Molienda: el Celecoxib fue molido con un molino de barra de impacto con discos contrarrotativos. A velocidades de molino entre unas 9060rpm / 5600rpm y unas 11200rpm / 5600rpm (rpm rotatorias/rpm contrarrotatorias) el tamaño de la partícula cambió dentro de rangos relativamente estrechos (por lo menos 90% de las partículas eran de 30 micrones o menos) sugiriendo*

*que la velocidad del molino no es estrechamente crítica para el proceso de micronización del medicamento a granel". (Página 59, renglones 12-17).*

Por lo tanto, nada de lo que dice este pasaje enseña o sugiere tamaños de partícula del celecoxib del rango descrito de unos 100nm a unos 900nm.

**WO 00/32189:** Este documento tampoco es relevante para la patentabilidad de la invención reivindicada y no hay ninguna revelación de celecoxib particulado (o algún otro inhibidor COX-2 selectivo) en el rango descrito de unos 100nm a unos 900nm.

En efecto, para temas diferentes a la novedad, la fecha efectiva de WO 00/32189 como referencia es su fecha de publicación, 8 de junio de 2000. Esta fecha es seis meses posterior a la fecha de prioridad de la solicitud de la referencia, que es 9 de diciembre de 1999. Por lo tanto, WO 00/32189 no constituye estado previo de la técnica para esta solicitud para efectos de determinar el nivel inventivo.

Ahora bien, si la revelación de WO 00/32189, se adujera como estado previo de la técnica, no convierte la invención reivindicada en una invención carente de nivel inventivo. En contraste con las nanopartículas descritas en las reivindicaciones pendientes, el grueso de la revelación de WO 00/32189 se dirige a las micropartículas del celecoxib:

*"En consecuencia, el tamaño de partícula D90 del celecoxib es preferiblemente de menos de unos 200mm, todavía más preferiblemente de menos de unos 75mm, aún más preferiblemente de menos de unos 40mm y muy preferiblemente de menos de unos 25mm... Además o alternativamente, el celecoxib tiene preferiblemente un tamaño promedio de partícula dentro del rango de unos 1mm a unos 10mm y más preferiblemente de unos 5mm y unos 7mm". (Página 30, renglones 11-18).*

Por lo tanto, WO 00/32189 no contiene una enseñanza ni sugiere que se reduzca el tamaño de partícula al rango de

la nanopartícula, es decir, al rango entre unos 100nm a unos 900nm, como se describe en la reivindicación 1.

Las sociedades opositoras no han citado ningún otro documento que contenga dichas enseñanzas. Ciertamente, sólo la especificación presente proporciona un vínculo entre el tamaño de la partícula y la biodisponibilidad de los medicamentos inhibitorios de la ciclooxigenasa-2. Dado el efecto impredecible de la reducción del tamaño de partícula sobre la biodisponibilidad, no se puede inferir tal vínculo.

Tal como lo señala el apoderado de las sociedades opositoras, cada solicitud de patente deber ser examinada por separado. Dado este efecto impredecible sobre la biodisponibilidad de la reducción del tamaño de partícula del medicamento demostrada por las referencias arriba analizadas, las preparaciones reivindicadas, así como los métodos de uso de las preparaciones, tienen nivel inventivo.

6.1.4 Por lo anterior y teniendo en cuenta el concepto de los términos novedad y nivel inventivo que establece la Decisión 486 de la Comunidad Andina, como requisito de patentabilidad, se debe señalar que las publicaciones WO00/53149 y WO00/32189 no pueden ser tenidas en cuenta como anterioridades que afecten la novedad de la invención en estudio, pues su publicación ocurrió el 14 de septiembre de 2000 y el 8 de junio de 2000, respectivamente, esto es en fechas posteriores a la fecha de la solicitud de la cual se invoca prioridad en este asunto (9 de diciembre de 1999).

En consecuencia, las publicaciones WO00/53149 y WO00/32189 no afectan la novedad del invento de que trata este asunto. Por tanto, se concluye que contrario a lo afirmado por la opositora, la presente invención sí es

novedosa de acuerdo con lo previsto en el artículo 16 de la Decisión 486 de la Comisión de la Comunidad Andina.

- 6.1.5 De otro lado, en cuanto al nivel inventivo la Decisión 486 en su artículo 18 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"*.

Como lo establece el artículo 16 de la Decisión 486, el estado de la técnica es todo aquello 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.

Ante lo anterior resulta claro que los documentos WO00/32189 y WO00/53149 que se aducen en contra de la patente de mi representada no habían sido publicados al momento de presentar la solicitud estadounidense 60/169.856, de la cual se reivindicó prioridad en este asunto.

- 6.1.6 De esta manera, se concluye que contrario a lo señalado por las sociedades opositoras 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:

Los opositores alegan 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, párrafo 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. y LABORATORIO FRANCO COLOMBIANO LAFRANCOL 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 12482-234-001-2  
COLO 12482-801-002-3  
JGG / ID.88



**Industria y Comercio**  
SUPERINTENDENCIA

Espacio reservado para el adhesivo de radicación

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**FORMULARIO UNICO DE CORRECCIONES Y MODIFICACIONES  
2000-10**

|                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                       |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b>1</b> SOLICITUD DE:</p> <p><input type="checkbox"/> Corrección de Error Material <input checked="" type="checkbox"/> Modificación a una solicitud</p>                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                       |
| <p><b>2</b> SOLICITANTE</p>                                                                                                                                                                                                                                                                               | <p>Nombre: <b>PHARMACIA CORPORATION</b><br/>sociedad constituida y existente bajo las leyes del Estado de Delaware, Estados Unidos de América</p> <p>Dirección: 5200 Old Orchard Road, Skokie, Illinois 60077, Estados Unidos de América</p> <p>Teléfono: 546 09 70<br/>E-mail: clientes@camyp.com<br/>Fax: 249 40 70<br/>Domicilio: Skokie, Illinois, Estados Unidos de América</p> | <p><b>IDENTIFICACION</b></p> <p>C.C. <input type="checkbox"/> NIT <input type="checkbox"/><br/>C.E. <input type="checkbox"/> Otro <input type="checkbox"/><br/>Número: _____</p>                                      |
| <p><b>3</b> REPRESENTANTE APODERADO</p>                                                                                                                                                                                                                                                                   | <p>Nombre: Emilio FERRERO</p> <p>Dirección: Carrera 4ª No. 72-35<br/>Teléfono: 347 38 11<br/>E-mail: cavellier@cavellier.com<br/>Fax: 217 92 11<br/>Domicilio: Bogotá, Colombia</p>                                                                                                                                                                                                  | <p><b>IDENTIFICACION</b></p> <p>C.C. <input checked="" type="checkbox"/> NIT <input type="checkbox"/><br/>C.E. <input type="checkbox"/> Otro <input type="checkbox"/><br/>Número: <u>438.019</u> TP <u>31.929</u></p> |
| <p><b>4</b> Número de expediente: <u>00.093.233</u> Clase: <u>C07C 143/080</u></p> <p>Título del Invento: <b>COMPOSICIONES INHIBIDORAS DE CICLOOXIGENASA-2 QUE TIENE RAPIDO ACCESO DE EFECTO TERAPEUTICO</b></p>                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                       |
| <p><b>5</b> Descripción de la corrección o modificación solicitada:</p> <p>De conformidad con lo previsto en el artículo 43 de la Decisión 486 de la Comisión de la Comunica Andina, presento <u>nuevo capítulo reivindicatorio</u>, el cual solicito sea tenido en cuenta.</p>                           |                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                       |
| <p><b>6</b> Comprobante de pago No. <u>04 - 21,254</u> Fecha <u>15 de marzo de 2004</u></p>                                                                                                                                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                       |
| <p><b>7</b> Anexos:</p> <p><input checked="" type="checkbox"/> Comprobante de pago de la tasa por \$ 87.000.00</p> <p><input type="checkbox"/> Poderes, si fuere el caso</p> <p><input type="checkbox"/> Certificado de existencia y representación legal, cuando el solicitante sea persona jurídica</p> |                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                       |
| <p><b>8</b> NOMBRE: <u>EMILIO FERRERO</u></p> <p>FIRMA: <u>Emilio Ferrero</u><br/>C.C. <u>438.019</u> de Usaquén T.P. <u>31.929</u></p>                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                       |

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Dependencia: 2020 DIVISION DE NUEVAS CREACIONES  
Folios: 88  
NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 04 - 21,254  
FECHA : MARZO 15 DE 2004

\*\*\*\*\* CONSIGNACION \*\*\*\*\*

| DEPOSITANTE       | TIPO PAGO    | BANCO         | CUENTA      | No. PAGO | FECHA PGO  | Vr. PAGO      |
|-------------------|--------------|---------------|-------------|----------|------------|---------------|
| CAVELIER ABOGADOS | CONSIGNACION | BANCO POPULAR | 050-00110-6 | 20338564 | 15/03/2004 | 35,966,000.00 |

\*\*\*\*\* CONCEPTO \*\*\*\*\*

| CANT. HISTORICO                              | CONCEPTO                      | TOTAL CONCEPTO |
|----------------------------------------------|-------------------------------|----------------|
| 1 30005-01-03 CAMBIO DE MODALIDAD Y MODIFICA | 12 MARCAS Y LEHAS COMERCIALES | 87,000.00      |
|                                              | TOTAL :                       | 87,000.00      |

SON: OCHENTA Y SIETE MIL PESOS

RESPONSABLE : \_\_\_\_\_

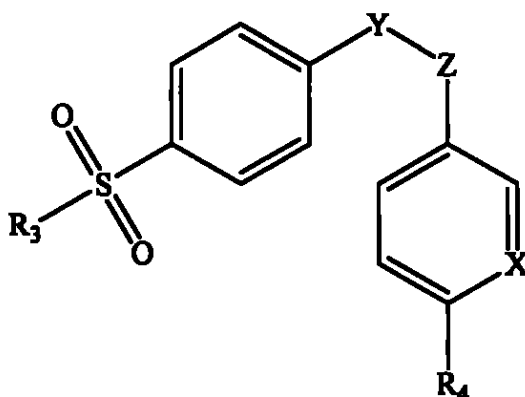
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Cdo 12482-234-001-2

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# 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

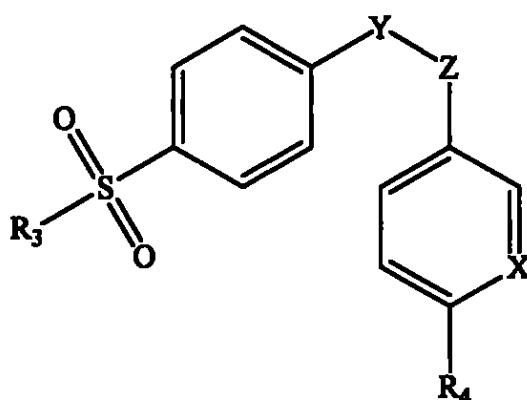
en la cual  $X$  es NO o  $CR_5$  donde  $R_5$  es hidrógeno o halógeno, y

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

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# REIVINDICACIONES

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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

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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-

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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.

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

Marilyn N. Martinez, PhD, and Gordon L. Amidon, PhD

This article provides an overview of the patient-specific and drug-specific variables that can affect drug absorption, including and protein metabolism. The rate and extent of drug absorption are determined by a complex spectrum of variables. Factors influencing drug absorption include drug solubility, permeability, and the rate of its dissolution. In this regard, the Biopharmaceutics Classification System has been used to be an important tool for predicting compounds likely to be associated with bioavailability problems. It also helps in identifying those factors that may affect the rate and extent of drug absorption. Patient bioavailability may also be markedly influenced by patient variables such as the integ-

egrity of the gastrointestinal tract, physiological status, site of drug absorption, metabolic competence, presystemic drug metabolism (first-pass metabolism), and metabolic variables such as the effect of food or concomitant medications. Through an overview of a drug's physicochemical properties and the physiological processes affecting drug absorption, the clinical pharmacologist can develop formulations that will maximize product bioavailability. By appreciating the potential impact of patient physiological status, phenotype, age, gender, and lifestyle, drug developers can be better informed to better meet the needs of the individual patient.

Journal of Clinical Pharmacology, 1998, 38(2):229-245  
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A fundamental principle associated with the use of a biopharmaceutical agent is that for any given patient, the clinical response can be predicted on the basis of the patient's drug product, dose, and dosing regimen. This tenet provides the foundation for concepts of predictability and reproducibility. Reproducibility refers to an inherent relationship between a therapeutic outcome and the dose and extent of drug exposure. A physician will prescribe a particular product to a patient with an expectation of a predictable response.

Generally, the problem of drug movement from the site of administration to the site of action can be characterized as follows (Figure 1):

As depicted in Figure 1, the relationship between drug intake and a clinical response is highly complex, potentially affected by a host of intrinsic and extrinsic

variables. Accordingly, deviations between drug response within or between individuals may be ascribed either to patient bioavailability (i.e., the rate and extent of drug absorption), drug pharmacokinetics (which includes the metabolism, distribution, and elimination of a compound), or the particular concentration-effect relationship.

While product formulation can significantly affect processes leading to drug absorption, once in the circulation, the original formulation is generally considered to no longer affect the ultimate drug response. In other words, it is the concentration of the drug moiety, along with its corresponding effect, that will ultimately determine product safety and effectiveness. For this reason, once a patient is treated to a particular product and dosing regimen, we assume that a comparable clinical response will be achieved if the patient starts to take a less expensive generic equivalent.

The purpose of this article is to discuss basic principles associated with the process of drug absorption. Special attention will be given to the use of the Biopharmaceutics Classification System (BCS) as a predictive tool for identifying compounds whose absorption characteristics may be sensitive to intrinsic

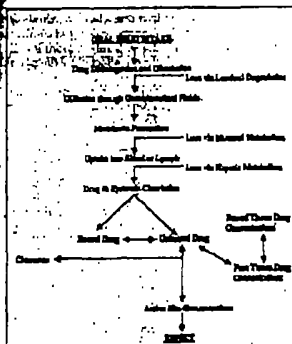


Figure 1. Schematic diagram of the relationship between the rate and extent of a drug product and its clinical effect.

(physiological) and extrinsic (e.g., food and concomitant) variables. Accordingly, this review focuses on those factors that can affect drug absorption, exposure, solubility, membrane permeability, and presystemic drug metabolism.

## WHAT IS THE BCS?

One of the most significant prognostic tools created to facilitate product development in recent years has been the BCS.<sup>1</sup> By knowing the solubility and permeability characteristics of specific compounds, we improve our ability to predict those variables (such as formulation, food, dosing regimen, and disease) that will alter oral drug absorption.

Currently, all pharmaceutical compounds are grouped into one of the following categories:

- Class I—high solubility, high permeability; generally very well-absorbed compounds
- Class II—low solubility, high permeability; exhibit dissolution rate-limited absorption
- Class III—high solubility, low permeability; exhibit permeability rate-limited absorption

Class IV—low solubility, low permeability; very poor oral bioavailability

Solubility is calculated on the basis of the largest amount manufactured. It is defined as the maximum solubility of drug across a pH range of 1 to 8 and at a temperature of 37 ± 0.5°C. High-solubility drugs are those with a ratio of dose to solubility volume that is less than or equal to 100 mL. Permeability ( $P_a$ ) is expressed in units of 10<sup>3</sup> cm per second and is defined as the effective human jejunal wall permeability of a drug. High-permeability drugs are generally those with an extent of absorption greater than or equal to 90% and are not generally associated with any documented bioavailability in the gastrointestinal tract.

It is interesting to note that for certain compounds,  $P_a$  is not necessarily constant. For example, nonlinear changes in  $P_a$  were observed with increasing doses of the histone-active molecule, flunitrazepam. This nonlinear increase in  $P_a$  was attributed to its effects on membrane vesicles taken up and to a possible decrease in poly-phosphatase (P-gp) activity associated with an increase in intestinal membrane fluidity.<sup>2</sup>

The application of this system to nonhuman species may require adjustment of these classification parameters based on physiological differences in gastric volume and the pH of the gastrointestinal (GI) fluids. Accordingly, at this time, we cannot be certain that the BCS classification of a compound remains constant across all species. This question is currently being explored by the Food and Drug Administration's (FDA's) Center for Veterinary Medicine.

By understanding the relationship between a drug's absorption, solubility, and dissolution characteristics, it is possible to define situations where *in vitro* dissolution data can provide a surrogate for *in vivo* bioequivalence assessments. The use of this surrogate relies on the validity of these fundamental assumptions. First, it must be assumed that a comparison of product *in vitro* dissolution performance accurately reflects relative differences in product *in vivo* dissolution behavior. Second, we must assume that if two products present with equivalent *in vitro* dissolution profiles under all relevant conditions, they will present equivalent drug concentrations at absorptive membrane surfaces. Third, for comparable absorption profiles to emerge comparable *in vivo* absorption, the rate and extent of drug presented to absorptive membrane surfaces must characterize the absorption characteristics of that drug product.

Leibenberg and Amidon<sup>3</sup> have summarized the relationships between dose, dissolution characteristics, drug solubility, and drug absorption properties. These relationships can be described as follows:

where  $M$  is the mass of the drug and  $V_d$  is the volume of distribution. The relationship between  $M$  and  $V_d$  is given by the equation  $M = V_d \cdot C$ , where  $C$  is the concentration of the drug in the plasma. The relationship between  $M$  and  $V_d$  is given by the equation  $M = V_d \cdot C$ , where  $C$  is the concentration of the drug in the plasma.

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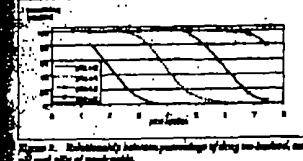


Figure 2. Relationship between percentage of drug absorbed and pH of weak acids.

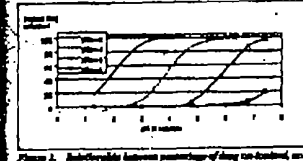


Figure 3. Relationship between percentage of drug absorbed and pH of weak bases.

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### IN VIVO DRUG ABSORPTION

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1. The first (intestinal) region contains a high proportion of water molecules and is the region responsible for absorption with other molecules and particles.
2. The second region has the highest molecular density of all four regions (contains the polar headgroups, contains little or no water, and serves the primary barrier to solute diffusion (due to its density characteristics).
3. The third region contains the highest density of nonpolar tails. This region serves as the primary barrier to nonpolar penetrating and hydrophobic compounds. As the thickness of the membrane increases, the density of the nonpolar tails increases, and the density of the water molecules decreases.
4. The fourth region is the most hydrophobic region of the membrane, serving as a hydrophobic barrier to molecular transport.

Owing to this membrane structure, it is evident that drug permeability is not a simply two-step process of solubilization and diffusion but rather represents a

spectrum of complex molecular events. Accordingly, intestinal permeability reflects multifactorial transport of drugs such as macromolecules (negatively charged), lipophilicity (positively charged), polar character (highly charged), and the molecular flexibility (intermolecular hydrogen bond formation).

In addition to passive mechanisms, barriers to drug diffusion, significant impediments to drug transport are the gastric and intestinal mucosa layers.<sup>10</sup> In assessing the relative contribution of these various components, it was noted that lipid components such as phospholipids, cholesterol, and small lipids (all lipophilic) reduced the diffusion of small lipophilic molecules such as paracetamol and hydrocortisone. Conversely, small hydrophilic molecules such as inulin appear to diffuse through the lipid barrier without any hindering components, such as cholesterol and phospholipids. However, they may serve to inhibit the diffusion of peptides and proteins.

Molecular flexibility and the corresponding ability to undergo conformational changes can significantly affect the polar surface area of a molecule. The polar surface area and molecular surface area are powerful predictors of intestinal permeability, being especially relevant for hydrophilic molecules. However, they may not adequately predict membrane permeability across the lipid bilayer component. Another important variable is the strength of the hydrogen bonds formed between the molecule and water and lipids.<sup>11</sup> It is generally accepted that these bonds must be broken (dissociated) before the solute can traverse the biological membrane, which of these factors play the dominant role in determining drug permeability are very much unknown.

Despite these complexities, some generalizations can be made with regard to drug absorption processes. For example, the vast majority of orally administered drugs are absorbed via passive transcellular transport.<sup>12</sup> This means that the drug traverses through a highly lipophilic membrane. Accordingly, diffusion processes are governed by Fick's laws of diffusion and therefore influenced by the compound's lipophilicity. This ability to diffuse through lipids has been found to be highly correlated with the ability of a drug to partition between water and an organic solvent such as octanol. In fact, when expressed as  $\log P$  (based on partitioning between *n*-octanol and water), the optimal partition coefficient for drug generally falls within the range of 1 to 7.<sup>13</sup> Nevertheless, exceptions do occur, and while transcellular transport generally occurs when

the compound is un-ionized, recently, several ionized molecules have been shown to be absorbed via transcellular pathways.<sup>14,15</sup> This finding reinforces earlier statements regarding the degree of ionic association with the relationship between  $P$  and drug absorption.

In addition to passive mechanisms, active transport is important in the absorption of several compounds. Both active and passive transport mechanisms may occur simultaneously for the same molecule. Which of these mechanisms has the dominant role tends to be improved specific and may not be well predicted by *in vitro* systems.<sup>16</sup> Nevertheless, it must be remembered that even active transport mechanisms require that the drug penetrate the intestinal cells via the transcellular route.

The rate of passive diffusion of any molecule, whether it be absorbed via transport between mucosal cells or through the mucosal membrane, can be described by the following equation<sup>17</sup>

$$\frac{dQ}{dt} = A_p \cdot \frac{D_p \cdot C_p}{L_p} = \frac{A_p \cdot D_p}{L_p} \cdot C_p$$

where

- $dQ/dt$  = the rate of passive drug absorption (amount of drug absorbed per unit time)
- $A_p$  = the diffusion coefficient of the compound in water
- $D_p$  = the square diffusion distance
- $L_p$  = the fluid flow between epithelial cells
- $C_p$  = the rate of the water-soluble drug in the solution phase (which is the influence of the solution phase, pH, and to a significant extent, the volume, shape, and hydrophobicity of the drug)
- $A$  = the permeability coefficient describing the ability tendency of the substance to diffuse in the membrane phase ( $A$  is not dependent on the representing aqueous phase  $C_p$ )
- $D$  = the diffusion coefficient of the compound within the membrane, which is dependent on factors such as drug lipophilicity, hydrogen bonding capacity, polar surface area of the molecule, molecular volume, and shape
- $L$  = the structural transport capacity of the membrane transporter (the Michaelis constant)

- $A$  = the thickness of the membrane
- $A_p$  and  $A_s$  = the available surface area for transcellular and paracellular transport, respectively

Thus, for passive diffusion, whether a drug is absorbed via paracellular or transcellular mechanisms is determined by both physicochemical and physiological factors.

Paracellular diffusion involves both diffusion and a convective volume flow through water-filled intercellular channels whose diameter is approximately 3 to 10 Å in humans.<sup>18</sup> Accordingly, the size and number of paracellular spaces influence the intestinal absorption of most hydrophilic compounds. This, in turn, is affected by the vascular surface area and by cellular density.<sup>19</sup> Therefore, it is not surprising that the permeability of small hydrophilic compounds tends to be greater in species such as rats, in which both pore diameter and surface area tend to exceed that in humans.<sup>20</sup> In humans, the small intestinal surface area for paracellular absorption is approximately 0.01% of the total mucosal surface area. Furthermore, unless the molecule is extremely small (e.g., < 200 Da), paracellular transport will have a negligible role in drug absorption.

With permeability-limited absorption, we can expect that although the fraction of dose absorbed remains unchanged, the absolute amount of drug absorbed will increase as dose increases (assuming linear kinetics). Conversely, for solubility-limited compounds, increasing the dose will have no effect on the absolute amount of drug absorbed. Consequently, in these cases, the fraction of dose absorbed will decrease as dose is increased.<sup>21</sup> Moreover, permeability is not necessarily constant throughout the GI tract. While for some compounds, drug absorption appears to be site independent,<sup>22</sup> for others, it is site dependent.<sup>23</sup> When drug absorption is site dependent, the availability of dissolved drug at the absorption site can be the rate-limiting factor in product bioavailability.

#### PRODUCT DISSOLUTION

Drug absorption depends on delivery of the drug particles to the site of absorption. The Noyes-Whitney equation describes the variables that can affect drug dissolution:<sup>24</sup>

$$\frac{dQ}{dt} = \frac{D \cdot S}{V \cdot L} (C_s - C)$$

where  $dQ/dt$  is the dissolution rate, expressed as the change in the amount of drug dissolved (m) per unit time ( $t$ );  $D$  is the diffusion coefficient;  $S$  is the surface area;  $L$  is the thickness of the diffusion film adjacent to the dissolving surface;  $C_s$  is the saturation solubility of the drug molecule;  $C$  is the concentration of the dissolved solute; and  $V$  is the volume of the dissolution medium.

Upon integration, this equation can be expressed as follows:

$$m = \frac{D \cdot S}{V \cdot L} (C_s - C) t$$

and

$$K = S \cdot D \cdot L$$

The viscosity of the GI contents<sup>25,26</sup> as described by the following equation, can affect  $D$ , the diffusion coefficient:

$$D = (K_1 T) / (\eta C_p L)$$

where  $D$  is the diffusivity of a compound,  $K_1$  is the Boltzmann constant,  $T$  is the temperature,  $\eta$  is the solvent viscosity, and  $\eta$  is the viscosity of the diffusion medium.

As seen in the latter equation, increasing the surface area of a particle ( $S$ ) can enhance its dissolution rate.  $S$  can be increased by micronization, a process sometimes applied to poorly water-soluble compounds.<sup>27</sup> Also to be considered are particle size and density, which both inversely affect the dissolution rate.<sup>28</sup> Particle shape is also important in determining the dissolution behavior of a drug, and for many crystalline forms (particularly shapes of needles and platelets), shape and consequently dissolution behavior may change markedly as the particle dissolves.<sup>29,30</sup>

Particle properties can also affect the rate of GI transit, the latter being highly dependent on such properties as size, shape, and density. For example, in swine, about 30% to 40% of ingested food particles pass into the duodenum within 10 minutes in an adult pig. However, large particles (10 mm in diameter) are retained within the stomach for several days, with residence time increasing with particle density and length.<sup>31</sup>

A problem with the Noyes-Whitney equation is that there is an inherent assumption that  $S$  remains constant over time. Unfortunately, this assumption is incorrect, and the  $S$  of powders and immediate-release preparations tends to decrease as dissolution pro-



orally versus parenterally. Alternatively, we must also consider the possibility that the increase in bioavailability (bioavailability was not affected by the effect of the route of administration) may be due to a change in the rate of absorption. This latter possibility is more likely to have been operative was strongly suggested by the results obtained when intravenous administration vs. oral dose was administered at the same time. In the latter study, the rate of absorption was not affected, but the bioavailability was increased. This increase in bioavailability was not due to a change in the rate of absorption, but rather to a change in the extent of absorption. This is supported by the fact that the rate of absorption was not affected by the route of administration. This point is underscored by the following two equations:

$$AUC_{0-\infty} = \frac{F \cdot D}{CL} \quad (1)$$

$$AUC_{0-\infty} = \frac{F \cdot D}{CL} \quad (2)$$

where  $F$  is the fraction of drug absorbed,  $D$  is the dose, and  $CL$  is the clearance. In the first equation,  $F$  is the fraction of drug absorbed, and  $CL$  is the clearance. In the second equation,  $F$  is the fraction of drug absorbed, and  $CL$  is the clearance. The first equation shows that  $AUC_{0-\infty}$  is directly proportional to  $F$  and inversely proportional to  $CL$ . The second equation shows that  $AUC_{0-\infty}$  is directly proportional to  $F$  and inversely proportional to  $CL$ .

where  $F$  is the fraction of drug absorbed,  $D$  is the dose, and  $CL$  is the clearance. In the first equation,  $F$  is the fraction of drug absorbed, and  $CL$  is the clearance. In the second equation,  $F$  is the fraction of drug absorbed, and  $CL$  is the clearance. The first equation shows that  $AUC_{0-\infty}$  is directly proportional to  $F$  and inversely proportional to  $CL$ . The second equation shows that  $AUC_{0-\infty}$  is directly proportional to  $F$  and inversely proportional to  $CL$ .

with the consideration of an enzyme inducer or inhibitor. Using saturating doses of  $CYP_{2D6}$  substrates, it was demonstrated that both enzyme induction and inhibition will have a less profound effect on  $AUC_{0-\infty}$  as compared with  $AUC_{0-12}$ , regardless of whether the compound in question is a high- or low-clearance drug.

The latter study points to the importance of considering the effect of a potential site of drug metabolism. Substantial drug loss can occur via intestinal effects such as metabolism (both Phase I and Phase II) and degradation within the gut lumen.<sup>17,18</sup> The cytochrome P450 system constitutes the principal enzymes involved in the biotransformation of drugs and other substances.<sup>19</sup> Approximately 70% of the human liver P450 is accounted for by  $CYP_{2D6}$ ,  $CYP_{2C9}$ ,  $CYP_{2C19}$ ,  $CYP_{2D6}$ ,  $CYP_{2C8}$ , and  $CYP_{2C18}$ . While the total amount of P-450 in the human intestine is much less than that in the liver (10 pmol/mg intestinal protein vs. 300 pmol/mg liver protein, respectively), the intestinal enzymes are strategically placed to metabolize drugs before they enter the systemic circulation.

Examples of drugs subject to gut wall metabolism include nifedipine,<sup>20</sup> nifedipine,<sup>21</sup> and nifedipine.<sup>22</sup> Interestingly, several other Phase I metabolizing enzymes co-exist at the villus tip, providing a highly efficient mechanism for drug degradation.<sup>23</sup> Of particular importance is the synergistic function of  $CYP_{2D6}$  and cytochrome P450 3A4, which together are responsible for the active extrusion and subsequent metabolism of a wide variety of compounds.<sup>24</sup> The latter is discussed in more detail in the section of this review that focuses on membrane transporter systems.

Compounds may also be individually metabolized in the gut lumen by digestive enzymes or by activity of the microflora. An excellent example is the potential negative impact of microbial metabolism on the bioavailability of oral contraceptives. The oral contraceptive norgestrel is metabolized in the gut lumen by the microflora, resulting in a decrease in bioavailability. This is supported by the fact that the rate of absorption was not affected by the route of administration. This point is underscored by the following two equations:

When evaluating the impact of drug metabolism on product bioavailability, patient phenotype should be considered (see Lu et al.<sup>25</sup> Mendez et al.<sup>26</sup>). Of equal importance may be the potential impact of certain enzymes, hormones, and inflammatory processes on the activity of these enzymes.<sup>27</sup> In this regard, Dr. David A. Flockhart, MD, PhD, in the Division of Clinical Pharmacology at Georgetown University, has developed a list of drug-drug interactions that act as substrates, inducers, and inhibitors of the P450 enzymes. The database includes Medline links to pertinent references for each of these substrates.<sup>28</sup>

#### PERMEABILITY EFFECTS

By understanding the relationship between  $D_{0-12}$  and  $AUC_{0-12}$ , we can assess whether a product's bioavailability will be significantly affected by its permeability. In this regard, the following factors are considered:

- pH dependent solubility of the drug.
- drugs that form insoluble complexes with the GI contents.
- solubility in the GI tract.
- physicochemical properties of drug particles. For example, to enhance the rate of drug dissolution of Class II compounds, the drug particles may be micronized. While this generally increases particle surface area (and therefore drug dissolution), micronized particles may also have a greater tendency to form aggregates, thereby decreasing drug dissolution.

Further use of the Noyes-Whitney equation can be enhanced via the use of wetting agents that lower the surface tension of the dissolution medium. Examples include dispersing agents such as polyethylene glycol and polyvinyl pyrrolidone or surfactant agents such as cyclodextrins.<sup>29,30</sup> However, surfactant agents that increase drug water solubility are not without drawbacks. For example, surfactants may increase the rate of drug dissolution, but they may also increase the rate of drug degradation. This is supported by the fact that the rate of absorption was not affected by the route of administration. This point is underscored by the following two equations:

For drugs that exhibit high solubility, low intestinal permeability, it is intestinal permeability that is the rate-limiting step in drug absorption.<sup>31</sup> In the BCS system, these are considered Class II compounds. A con-

sequence of permeability-limited absorption is that the rate of dissolution is generally the rate-limiting step in the rate of GI uptake. Consequently, formulations of Class II compounds may exhibit markedly different rates of dissolution without affecting any differences in the blood concentration-time profile.<sup>32</sup> However, Class II compounds may be extremely sensitive to the potential impact of GI transit time and permeability.

Some compounds are known to exert direct physiological effects. For example, owing to its osmotic activity, sorbitol may alter GI transit time, resulting in more rapid product transit through the intestine.<sup>33</sup> Another compound known to alter GI transit time is sodium dodecyl sulfate.<sup>34</sup> The action of these agents could significantly reduce product bioavailability. Obviously, these compound effects would go undetected by traditional *in vitro* dissolution methods. Other examples of potential *in vitro* dissolution effects that could go undetected during *in vitro* dissolution testing include the effect of surfactants on membrane permeability (e.g., Dole et al.<sup>35</sup>) and the inhibition of other pumps.<sup>36</sup> Accordingly, one cannot necessarily view *in vitro* dissolution as a predictive tool for *in vivo* bioavailability. One can, however, use *in vitro* dissolution as a tool to assess the potential impact of these agents on drug bioavailability.

#### FACTORS THAT CAN AFFECT PRODUCT BIOAVAILABILITY

##### Food Effects

The effect of food on drug oral bioavailability is extremely complex, and the underlying reasons for food effects appear to differ with different agents. For example, in some cases, it appears that gastric content is an important variable contributing to the magnitude of the food effect. In other cases, gastric content appears to have minimal influence on drug bioavailability.<sup>37</sup> When drug absorption is food-limited, food may enhance drug bioavailability. In other cases, food may have negative effects on both the rate and extent of absorption as the drug binds to food constituents.<sup>38</sup>

Depending on the physicochemical properties of the compound, food-induced changes in gastric emptying, along with an increase in gastric pH, may increase, decrease, or have no effect on drug bioavailability.<sup>39</sup> For exam-

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ph. certain antineoplastic drugs are acid labile, and their absorption depends on the pH of the gastric juice. These drugs are most stable in the acidic environment of the stomach. In the small intestine, the pH is higher, and these drugs are rapidly degraded. Therefore, the degree of ionization of these drugs is a major factor in their absorption. The degree of ionization of a drug is determined by its pKa and the pH of the environment. The Henderson-Hasselbalch equation can be used to calculate the degree of ionization of a drug at a given pH.

**Effect of pH on GI Transit Time**  
The rate at which the drug passes through the GI tract is highly dependent on the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

- There is a significant effect of pH on the rate of transit through the GI tract.
- The pH of the stomach is low, while the pH of the small intestine is higher.
- The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

By increasing fluid volume, the gastric emptying rate is increased. This is because a larger volume of fluid in the stomach leads to a faster rate of peristalsis. The rate of gastric emptying is also influenced by the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

**The Effect of Motility on Drug Uptake Characteristics**  
The rate of drug uptake is influenced by the rate of peristalsis, which is determined by the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

Transit time in the GI tract is a major factor in drug absorption. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

Many drugs are absorbed in the small intestine. The rate of absorption is determined by the rate of peristalsis, which is influenced by the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

Small hydrophilic molecules are generally absorbed in the small intestine. The rate of absorption is determined by the rate of peristalsis, which is influenced by the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

Many recently, using two *in vitro* preparations, Caco-2 cell monolayers and the brush border membrane vesicles (BBMV), it was concluded that the permeability of the brush border membrane is a major factor in drug absorption. The rate of absorption is determined by the rate of peristalsis, which is influenced by the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

**Local Effects on Drug Diffusivity**  
The effect of local viscosity on drug absorption will depend on whether or not the drug is a small molecule. The rate of absorption is determined by the rate of peristalsis, which is influenced by the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

absorption, particularly if absorption is limited to the upper portions of the small intestine. If the drug exhibits site-specific absorption, then local viscosity will be a major factor in its absorption. The rate of absorption is determined by the rate of peristalsis, which is influenced by the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

When present, altered drug absorption associated with local viscosity appears to be attributable primarily to altered fluid flow dynamics rather than to altered GI motility. However, only modest changes in gastric emptying rate are observed when viscosity increases above 15,000 cP. Accordingly, viscosity-induced delays in gastric emptying are primarily seen when comparing changes that occur within the lower range of potential viscosity values. For this reason, viscosity effects on drug bioavailability are generally considered to be primarily a consequence of the effect of viscosity on GI transit time.

**The Effect of Motility on First-Pass Drug Metabolism**

Intestinal P450s are found in the brush border membrane of the small intestine. The rate of absorption is determined by the rate of peristalsis, which is influenced by the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

There are numerous examples of enzyme induction in the GI tract. These include the following:

- Chemical-induced and natural factors: These polycyclic aromatic hydrocarbons are potent inducers of CYP1A1 and CYP1A2. Examples of drugs whose bioavailability may be decreased because of their effects include phenytoin and theophylline.
- Grapefruit juice: An unclassified compound within grapefruit juice is recognized to inhibit both CYP2C9 and CYP3A4. As a result, there is increased bioavailability of compounds such as nifedipine, midazolam, and cyclosporine.

- Continuous variables: These are factors that are always present and are not subject to change. Examples include age, sex, and body weight.
- Discrete variables: These are factors that are present or absent. Examples include the presence of a disease or the use of a drug.
- Continuous variables: These are factors that are always present and are not subject to change. Examples include age, sex, and body weight.
- Discrete variables: These are factors that are present or absent. Examples include the presence of a disease or the use of a drug.

For drugs exhibiting a large first-pass effect, concomitant intake with food may increase drug availability. A classic example of this is propranolol, as a result of, in part, its substantial first-pass metabolism. A food-induced increase in hepatic drug delivery can lead to reduced presystemic metabolism. Another critical variable is the volume of fluid taken with the dose, whereby increasing the volume of fluid intake from 250 to 500 mL significantly increases the rate and extent of absorption bioavailability. This relationship appears to be attributable to the decrease in first-pass metabolism associated with a fluid volume-induced decrease in GI transit time.

There appears to be little correlation between the composition of a particular meal and the rate of drug absorption. This is because the rate of drug absorption is determined by the rate of peristalsis, which is influenced by the pH of the environment. The pH of the stomach is low, while the pH of the small intestine is higher. The rate of transit through the GI tract is determined by the rate of peristalsis, which is influenced by the pH of the environment.

For further information in this regard, an extensive list of metabolic pathways and associated information can be obtained through the World Wide Web.

**APPENDING BCS CONCEPTS TO PREDICT THE IMPACT OF PHYSIOLOGICAL STRESS AND FOOD ON PRODUCT BIOAVAILABILITY**

There are numerous examples where a priori knowledge of a compound's physicochemical properties and

NCE classification can help predict the biodegradability under a given set of conditions. For example, we have shown that compounds with poor aqueous solubility (such as styrenes and hormones) are often associated with absorption problems in subcutaneous implants.<sup>4,5</sup> Similarly, we would expect that the biodegradability of these compounds will be overpre-

GI transport there can also be stimulated by a number of factors, including age, food, drug, endocrine, disease, and stress, and even spans over the circadian clock. However, in this report, the ABC can be used to monitor GI motility in subjects whose bioavailability may be affected by these factors. In GI transit, the pH in the stomach can be obtained from the  $\text{H}_2\text{O}$  of GI contents (e.g.,  $\text{pH}$ ) and may be more transient with rapid GI transit (e.g.,  $\text{pH}$ ), than the stomach as compared to subjects with a longer transit time such as  $\text{pH}$ . Thus, we can predict that Class II compounds (high  $\text{pH}$  permeability, low solubility) that tend to be absorbed in the small intestine may be particularly sensitive to the intestinal absorption. GI transit time

likely sensitive to future cost increases in timber harvest. These factors alone may suggest a difference in *Pin* in the two Minnesota cities, indicating a positive relationship under certain sets of conditions between the two cities. Conversely, if the positive relationship is not significant, changes in the problem objective (timber harvest) for Chisholm City are not likely to be significant. The results of the *Pin* sensitivity analysis are shown in Table 10. Low permeability, high activity (1) responses are decreased (1) result time. In these cases, the 1) drop absorption, not discharging; that is the rate limiting step. The 1) permeability of the material will remain relatively unaffected. However, we see likely to see that a decrease in 1) *Pin* results in the rate limiting step being shifted, thereby affecting product permeability. Individuals have some of the other byproducts and oxidation appear to have significantly less oxidative response, it is likely that appears to be a decrease in the rate limiting step. The results of the sensitivity analysis are shown in Table 11. Therefore, these individuals are very potentially exhibit poor permeability of many City of Minnesota.

The construction of the GCM was based on the number of wells, minerals, weather probability, observed temperature, precipitation, stream flow, and field volume. The primary effect being measured by P-Flow was the possible change in precipitation across the length of the GCM tract, the gap was to be viewed as a complex heterogeneity. Additionally, data absorption processes are modeled in accordance with the assumption of three-layered processes (not correct). While this may be correct for many hydrological systems (e.g., Class I and III compounds), it is unlikely to be the case for drugs, that are absorbed throughout the GCM tract or the

chamber throughout the length of the small intestine. In the latter situation, dissolution and uptake processes are subjected to highly variable conditions, and the use of oral and rectal procedures is recommended.

The suggestion of these evidence is technically employed by the present study. While the *in vitro* absorption may adequately describe (S<sub>1</sub> + C<sub>1</sub>) compounds (as well as some Class II and IX compounds), it cannot adequately describe drugs with very low solubility and/or permeability. Therefore, the use of two *in vivo* models may be more appropriate for describing the absorption of certain Class I, III, or IV compounds.<sup>1</sup> It may also be useful to describe the effect of food on drug absorption, particularly since the impact of food on absorption and velocity decreases as the *pH* increases the small intestine ( $pH = 1-6$ ).

Finster et al.<sup>27</sup> provide an excellent review of the variables that can affect product bioavailability. In brief, some of the factors that can potentially affect oral drug bioavailability include the following:

11. A further fact was that the person who was the subject of the investigation was a member of the same family as the person who was the subject of the investigation.

- [illegible]

the kinetics or that are observed throughout the small intestine.

- Class I compounds are distinguished relative to their high peak and its GC characteristics
  - Class I compounds are, by definition, highly volatile, they occupy a lower stage of peak values. Therefore, they are not expected to affect the extent of product degradation. However, the delay in gastric emptying may affect peak drug concentrations or the time to reach peak drug concentrations.
- Class II compounds exhibit lower solubility and/or higher permeability. Accordingly, implying that one or more barriers are in vivo dissolvable will not be the case. The solubility of these drugs is low. Due to the low solubility of Class II compounds, high-dose meals (which cause increased GI motility and thereby inhibition) will generally enhance drug dissolution. This is particularly true for weak acids and weak bases exhibiting high peak values. However, weak bases with low pKa values may tend to precipitate in the stomach because of the pH changes when the pH of the GI contents is elevated, due to food.
- Class III compounds are characterized by high solubility and low permeability. Therefore, gastric emptying with drug permeability can markedly affect drug absorption. Many of these compounds are either hydrophilic or dependent permeability. Accordingly, while they will not resist drug dissolution, they are often absorbed (e.g., viscosity of stomach including a physical barrier to drug absorption). However, the mechanism of drug absorption is not the same for all drugs. It is possible to assess this class of compounds and determine if it is likely to be affected.

### MOLECULAR TRANSFORMATION

The fibrous disruption of a drug product is largely governed by its chemical composition, drug quality and purity, and the history of the lot on exposure to the air and to solvents. However, these are not the only factors. The amount of drug reaching the hepatic portal circulation is influenced with equal importance by these properties. Obstructions, computed. Morbidity characteristics (the greater or lesser time required) can be attributed to the breakdown of substance into various parts. This characteristic has led to a range of studies focusing on the identification and role of various components in modifying their pharmacokinetics in both human and veterinary species.

Transport proteins have been implicated in both influx and efflux processes across a host of tissues, including the intestine, kidney, liver, and the central nervous system.

your system. <sup>22</sup> Moreover, genetic variations in transporter gene sequences have been implicated in several severe genetic disorders. For this reason, Yan and Shuler<sup>23</sup> have developed a Web-accessible interactive human molecular transporter database that contains information such as genetic sequence, gene family, transporter structure, function and substrate, tissue distribution of the transporter, and genetic disorders associated with transporter sequence variants.

- In general, membrane transporter activities tend to fall within the following classifications:
  - Facilitative transporters passive transporters that assist the passage of a single solute (unipporter) down an electrochemical gradient.
  - Active transporters transporters that move solutes against an electrochemical gradient and therefore must be coupled to the generation of the energy.
    - Ion pumps/anti-transporters coupled to an energy-yielding chemical or a photochemical reaction. In some cases, these ion pumps result in a net movement of charge, generating a voltage difference across the membrane.
    - Secondary active transporters use the voltage and ion gradients generated by the primary active transporters.
      - Cotransporters symporters move two or more different solutes in the same direction.
      - Antiporters move two different solutes in opposite directions, exploiting an external gradient to drive the action.

Table 1 contains examples of transporter families and the substrates whose transmembrane absorption is believed to be linked in some manner to a carrier-mediated mechanism.

The intestinal location of these transporters appears to be site specific. For example, transporter-mediated iron absorption occurs primarily within the upper duodenum while vitamin B<sub>12</sub> absorption occurs in the terminal ileum.<sup>40</sup> Additional specificity is conferred by several tissue differences in transporter distribution.<sup>41</sup>

While the vast majority of transporters are located on apical membrane surfaces, some exist primarily on basolateral membrane surfaces. The latter include the Na<sup>+</sup>/K<sup>+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> ATPases, Na<sup>+</sup>/HCO<sub>3</sub><sup>-</sup> cotransporters, Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> cotransporters, GLUTs, Na<sup>+</sup>/glucose cotransporters, and Na<sup>+</sup>/independent amino acid transporters. In addition, others may be found on either membrane surface, including K<sup>+</sup> channels, Na<sup>+</sup>/K<sup>+</sup> ATPases, Na<sup>+</sup>/HCO<sub>3</sub><sup>-</sup> cotransporters, and an Na<sup>+</sup>-independent phosphate transporter.

Some drugs act as a substrate for multiple transporter systems, as is the case with several anticancer drugs.<sup>24</sup> In these situations, absorption is compli-

**Think of the questions and Think of the answers.**

[illegible]

ected by drug affinity for both intrins and extrins mechanisms. Therefore, any number of factors may be capable of influencing drug bioavailability.

Drug absorption has been associated with a wide range of substances and a host of potential transporters. Active systems have been implicated for the intestinal mucosal transport of organic cations (such as norepinephrine,  $\beta$ -alanine, and  $\beta$ -aminoacids), organic anions (glucuronide conjugates), presumably vital nutrients, small molecules (ATP transporters, nucleotides), paracrine factors, and certain polar peptides. The regulated secretion of Phase II metabolites may also be subject to binding and transport via the basolateral carrier substance proteins (MCTs). An ATP-binding cassette "P-glycoprotein" responsible for extruding compounds such as topoisomerase inhibitors and some of the anti-tumor drugs is

Due to their broad range of substrate specificity, much of the recent research has focused on P-gp and the glycoprotein transporter, Pgp1. Accordingly, the remaining portion of this review will concentrate on these two transporter systems.

1992

The acidic pH generated by the brush-border Na/H exchanger serves as the driving force for transmembrane absorption of aldehydes, triglycerides, and peptide drugs.<sup>2,3,4</sup> However, while penetration of the apical membrane appears to depend on an apical Na<sup>+</sup>/lipid cotransporter (Fig. 1), after entering the enterocyte, these proteins that are resistant to hydrolysis by intracellular proteolysis are transported across the basolateral mem-

brane by an H<sup>+</sup>-independent transporter, possibly PspT.<sup>24</sup> PspT is thought to exist on the apical membrane while the related PspA is thought to be located on the basolateral membrane surface.<sup>24</sup>

The density of the  $\text{C}_{60}$  molecules from the thorosphere to the Geom, being most abundant at the 90th tip. It is supposed to represent hypothetical states, increasing in the presence of thorium. It is also relatively measured by a variety of data (such as  $\text{C}_{60}$ -induced) that are adversely affected by the location of other thorium systems. This observation has raised the suggestion that large whole-sphere systems related to the  $\text{C}_{60}$  may exhibit low variability during a course of density that would encompass where thorium is deposited with an explicit thorium concentration.

PeptT1 possesses two, positive sites for phosphorylation by protein kinase C, with protein kinase C activation suppressing peptidic transport activity to 50–60% of control. PeptT1 is also negatively affected by an increase in the level of cAMP, presumably a consequence of a cAMP-induced stimulation of protein kinase C.

Certain Pepti substrate structure-activity relationships have been identified. Accordingly, while some exceptions do occur (e.g., several angiotensin-converting enzyme inhibitors), most substrates exhibit the following features:

- a free O-terminated carboxyl group or a group capable of hydrogen bond formation.
- an anion group or a weakly basic group at the N-terminus.

- g. highest affinity is associated with molecules that enter as substrates,

Compounds absorbed via the activity of dipeptide transporters exhibit saturable absorption. Consequently, substrate intestinal permeability ( $P_a$ ) can generally be described by one of the two following equations:<sup>24</sup>

$$P_{ij} = L_{ij} / (X_{ij} + C_{ij}) + P_{ij}$$

$$P = P_{\text{Л}} + C_{\text{ВЛ}} + P_{\text{В}}$$

where  $J_{\text{max}}$  is the maximum transport rate,  $C_e$  is the intestinal wall concentration,  $K_m$  is the molar concentration of substrate at  $1/2 J_{\text{max}}$ ,  $P_c$  is the permeability defined by the carrier-mediated transport, and  $P_u$  is the intestinal permeability attributable to passive diffusion.

[illegible]

While traditional efforts to increase drug bioavailability have focused on drug solubility and permeability, recent strategies have focused on the development of prodrugs as a strategy for improving drug absorption. By targeting broad-specificity transporters such as Pgp1, drug absorption can be markedly enhanced.<sup>24</sup> Once transported into the enterocyte, cytosolic enzymes convert the prodrug to its active form.<sup>25</sup> Thus, by developing formulations that act as the inherent physiological stimulator of the enterocyte, the absorption of otherwise poorly bioavailable compounds can be markedly improved.

### ATP-Binding Cassette Transporter Protein

Another family of proteins whose impact on drug absorption is currently under intense investigation is the ATP-binding cassette (ABC) transporters. Based on conserved sequences, there are estimated to be approximately 48 ABC transporters in humans. Mutations of these genes have been associated with the development of disease such as cystic fibrosis and obstetrical cholestasis. Overexpression of these transporters has been implicated in various drug-resistance states.<sup>12</sup>

Amphibolite of ABC transporters in the multilayered resistance proteins (MRP). The MRP-associated gene family consists of seven members MRP1, MRP2, MRP3, MRP4, MRP5, MRP6, and MRP7.<sup>1,2</sup> Numerous assays may be applied to the same paradigm. For example, MRP1 = ABCC1 = MRP; MRP2 = ABCC2 = cMOAT (phenol/bilirubin glucosyltransferase); MRP3 = ABCG2 = MDR3; MRP4 = ABCG4 = MDR4; MRP5 = ABCG5 = MDR5; MRP6 = ABCG6 = MDR6; MRP7 = ABCG8 = MDR8. These transporters differ both in terms of substrate specificity and in primary location within the body.<sup>3</sup>

In this part, the  $\text{MMP-2}$  gene product located at the apical surface membrane,  $\text{F-gp}$ , serves as an effective barrier to the intestinal absorption of numerous substances.<sup>10,11</sup> Evidence also shows a highly effective mechanism for transporting a variety of substances from the blood back into the intestinal tract.<sup>12</sup>  $\text{F-gp}$  is located on the apical surface of many organs, including the bladder, kidney, brain, liver, lungs, pancreas, stomach, spleen, synovium, and the large and small intestine.<sup>13,14</sup> In the intestine, the ratio of  $\text{F-gp}$  from the basolateral to apical versus the apical to basolateral direction ranges from 1.4 to 11.8, depending on location within the GI tract.<sup>15</sup>

**Fig. 2.** **Fig. 2** shows AIT-derived energy to fuel the entire effort of its substrate. Although not the sole energy-dependent recovery mechanism affecting drug bioavailability,<sup>12</sup> **Fig. 2** appears to have the most widespread importance due to its high intestinal concentrations, its location within the intestine, and its broad substrate specificity. **Fig. 2** is located on the villus tip of the apical brush-border membrane, with minimal or no levels found within the intestinal crypts.<sup>12</sup> Its activity tends to increase progressively from the stomach to the colon in humans,<sup>12</sup> a consideration when developing sustained-and/or delayed release formulations.

ABC transporter provides share a common nucleoside structure. This includes two large transmembrane domains (forming a pathway through which the solutes

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move) and two anionic sites. The transporters are located in the brush border and apical membrane of the cells. The transporters are located in the brush border and apical membrane of the cells. The transporters are located in the brush border and apical membrane of the cells.

The transporters are located in the brush border and apical membrane of the cells. The transporters are located in the brush border and apical membrane of the cells. The transporters are located in the brush border and apical membrane of the cells. The transporters are located in the brush border and apical membrane of the cells.

Upon studying the structure-activity relationship of P-gp substrates, several points were noted.<sup>10</sup> First, membrane partitioning is the rate-limiting step in the binding of substrates. Accordingly, hydrophobic substrates play a predominant role in membrane partitioning. However, numerous hydrophilic molecules are also actively transported, most probably reflecting the multiple potential points of entry to this

transporter protein. Substrate affinity does not appear to be affected by molecular charge since there are examples of neutral, positively charged, and negatively charged molecules that are effectively cleared by this transporter.<sup>11</sup>

Second, a compound must possess the ability to undergo hydrogen bonding with the transporter as a mechanism for activating the ATPase. The number and strength of these hydrogen bonds determines the dissociation of the P-gp-substrate complex. The binding of compounds with a high number of hydrogen bond acceptor groups can lead to a lowering of the ATPase activation. Accordingly, the rate of hydrolysis appears to be inversely proportional to the number and strength of hydrogen bond acceptor groups per compound. If two substrates are structurally similar, the P-gp, the compound with the higher potential hydrogen bond with P-gp will not be as effective for its clearance.<sup>12</sup>

Third, structures are markedly enhanced by the co-location of metabolizing enzymes and multidrug-resistance proteins.<sup>13,14</sup> Cytochrome P450 (CYP) 2A and P-gp are often co-located on the apical surface of small intestine villi, exhibiting overlapping substrate specificity and shared inducers and inhibitors. Via their co-located activities, drugs that are CYP2A substrates are continually cycled between the enterocyte and the gut lumen, thereby maintaining the extent of drug exposure to the metabolizing enzyme. Upon P-gp inhibition, the drug undergoes only a single exposure to the enzyme, leading to a decrease in drug degradation and higher oral bioavailability. However, despite their closely aligned relationship, there appears to be independent genetic regulation of these proteins, as evidenced by the lack of correlation in the magnitude of the phenotypic expression of CYP2A and P-gp across individuals.<sup>15</sup>

Considering the potential impact of P-gp and CYP2A activity on drug bioavailability, Benet et al.<sup>16</sup> observed that the fraction of drug absorbed could be predicted by the following equation:

$$F_{rel} = F_a \cdot F_g \cdot F_m$$

- where
- $F_{rel}$  = the oral bioavailability of a compound,
- $F_a$  = the fraction of the oral dose that gets absorbed,
- $F_g$  = the fraction of a dose that passes through the gut into the hepatic portal blood without being metabolized.  $F_g$  can also be described as 1 -  $f_{hep}$ , where  $f_{hep}$  is the gut metabolism ratio,
- $F_m$  = the fraction of a dose that is not metabolized

on first pass through the liver.  $F_g$  can also be described as 1 -  $f_{hep}$ , where  $f_{hep}$  is the hepatic metabolism ratio.

$$F_{rel} = F_a \cdot F_g \cdot F_m$$

$$F_{rel} = F_a \cdot F_g \cdot F_m$$

where  $k_a$  is the absorption rate constant for the drug entering the blood from the intestine,  $k_{el}$  is the rate constant for drug metabolism in the gut,  $Q_{hep}$  is the hepatic blood clearance, and  $Q_{cl}$  is the hepatic blood flow.

Ultimately,  $F_{rel}$  can be modified by numerous exposure to substances that act as inducers or inhibitors of these metabolic processes. P-gp inhibitors and inducers act by altering  $k_a$ . The changes of a corresponding change in  $f_{hep}$  can be explained by the constant change in substrate elimination from the systemic circulation.<sup>17</sup>

As evidenced by drugs that are metabolically inert such as talazoparide<sup>18</sup> and benzocaine,<sup>19</sup> P-gp contributes to drug-dependent oral bioavailability such that bioavailability can increase solely due to active pump inhibition. In addition, intestinal absorption of substrates can be increased through substrate competition, ATP depletion, or changes in membrane fluidity. Numerous inhibitors such as verapamil, quindine, and nifedipine have also shown to increase the bioavailability of P-gp substrates such as digoxin, fentanyl, and verapamil.<sup>20</sup> Conversely, P-gp inducers such as rifampin can decrease the bioavailability of certain substrates by increasing total gut activity of the efflux protein.<sup>21</sup>

P-gp inhibition may be most clearly seen by the effect of the genetic mutation. In mice, the inhibitor gene type results in "knockout" mice that exhibit a 100% decrease of gut P-gp activity. These mice were used to examine the P-gp role in limiting the intestinal absorption of fentanyl.<sup>22</sup> Fentanyl AUC values after oral administration in wild-type mice (homozygous) versus knockout mice (homozygous) were 13% and 35%, respectively, indicating secretion following intravenous administration was practically unchanged in knockout mice, even though 60% of the dose underwent intestinal secretion in the wild-type mice. Shorter distances in wild-type versus knockout mice bioavailability were observed for such compounds as verapamil, digoxin, talazoparide, and talazoparide.<sup>23</sup> An effect corresponding to the P-gp gene was noted in mice as well as observed in humans, where certain variants in the MDR1 gene have been shown to alter both the gut permeability of P-gp and the oral bioavailability of P-gp substrates.<sup>24</sup>

Permeability enhancers such as the bile salt sodium deoxycholate,<sup>25</sup> fatty acids such as sodium caprylate,<sup>26</sup> and surfactants<sup>27</sup> such as polyoxyethylene 40, 60, 90, and 150 can alter P-gp activity. The mechanism by which these substances interact with P-gp appears, at least in part, to involve a change in membrane fluidity.

Dedeja et al.<sup>28</sup> provided data suggesting that surfactants such as Brijol H-10, Tween 40, and Cremophor EL decrease lipid fluidity of both intact cells and isolated crude plasma membranes. Similarly, the ATPase activity of the membrane vesicles is decreased in the presence of lower membrane fluidity.<sup>29</sup> Furthermore, membrane vesicles from Chinese hamster ovary cells were shown to exhibit lower membrane fluidity than did their drug-sensitive counterparts.<sup>30</sup>

In contrast, membrane fluidity was found to increase in the presence of other fatty acids, Tween 80, Triton X-200, and verapamil, and this increase in fluidity was correlated with the inhibition of P-gp ATPase activity in multidrug-resistant hamster ovary cells.<sup>31</sup> Moreover, the bile salt, taurochenodeoxycholate, and verapamil increased membrane fluidity and decreased P-gp activity in multidrug-resistant hamster ovary cells.<sup>32</sup>

Although the reasons for these discrepancies are not obvious, each of these studies consistently noted that surfactants would alter drug efflux. The combination of efflux transporter inhibition, enhancement of membrane diffusion, and facilitation of paracellular diffusion may be a substantially increased oral drug bioavailability. Consequently, formulations are developing the use of these and other P-gp inhibitors as a formulation strategy for improving the absorption of poorly bioavailable compounds.

#### Other Transporter Systems of Interest

##### Organic Anions and Cations

An amphiphilic anion transporter family with the capability of transporting organic anions and cations has been recently defined.<sup>33</sup> Although their full scope of functional significance has yet to be determined, members of this family are diverse, exhibiting a number of characteristics, including the following:<sup>34</sup>

- a sodium-dependent transmembrane transporter;
- a sodium-independent and potential-dependent transporter that mediates absorption of pyrimidine, pyridine, and thiopyridine

...and potential independent transportation that  
...the transportation of children and may be by  
...in the interests of expediting them through an  
...of the national highway system.

1. Proteinuria - presence of protein in the urine. It is a common finding in many renal diseases and is often a sign of glomerular damage. It can be detected by a urine dipstick test or by a more sensitive test called a 24-hour urine collection.

Special Criminal  
Transportation

Allyl of interest to the chemist is the allyl group. With compounds containing long chain fatty acids (LCFA) and cholesterol, chemical analysis of the allyl group in the diglyceride component has been used to identify individual fatty acids. Substrates containing weak hydroxyl functions, generally by hydroxyl groups, are converted to the allyl form in the stomach (the high lipophilicity of these compounds might allow substrates to escape directly by passive diffusion). However, recent evidence suggests that (although cholesterol and LCFA are suggested to be digested through the enterocyte membrane, these compounds exhibit suitable absorption, relatively transport proteins are concentrated in the stomach and on the villi, etc. of the distal small intestine of humans). Fats are consistent with earlier findings that substances such as cholesterol appear to be absorbed via a noncarrier-mediated process of low concentration and by passive diffusion after esterification (as occurred in cholesterol). Although, the several binding proteins influencing the transfer of cholesterol compounds.

[illegible]

In addition to metal-mediated processes, chlorophyll absorption also appears to be affected by the action of metal-binding-resistant stress proteins, the latter appearing to be the same transporter responsible for mobilizing the chlorophyll of plant stress.<sup>10</sup> It was noted that two main studies now in ABC transporter genes are responsible for the development of stress tolerance. Two of these genes are expressed at highest levels in the root and shoot systems and appear to be involved in stress-associated gene expression.

the development of *athandromimids*.<sup>20</sup> Moreover, animal model studies reveal the impact of genetic mutations on the presence of abnormal cholesterol and bile salt metabolism.<sup>21</sup> Therefore, given the growing recognition of the importance of transporters in maintaining cholesterol homeostasis, it is evident that these transporters are becoming a target for new drug therapies.

### CLOSING STATEMENT

**Drug absorption** is a complex process that involves the physical properties of a drug, its variability, both physicochemical and physiological. The widest scientific chemist activity manipulates product permeation by the use of manufacturing methods and product design to increase the solubility and permeability of problematic compounds. However, physiological variables, both endogenous (such as age, sex, diet, genotype, and genetic) and exogenous (such as food consumption, disease, concomitant medication, and environmental exposure to other xenobiotics), can markedly affect the absorption characteristics of a compound. By understanding the physicochemical properties of a compound (such as pKa, log P value, molecular flexibility, hydrogen bonding dynamics, etc.) and the physiological processes that govern its pharmacokinetics (e.g., gastric emptying time, intestinal transit time, and mucosal permeability), we can predict, with some degree of accuracy, a patient's expected bioavailability. Thus, through awareness of a drug's NCI characteristics and the physiological processes affecting drug absorption, the pharmaceutical scientist can better predict drug absorption and develop accordingly his formulation to be applicable when considering the drug's target cellularity in the face of varying patient physiology (age, race, phenotype, sex, genetics, etc.).

With respect to vesicular transport, the cell membrane and the factors that govern their activity have been the recent focus of much research activity. Accordingly, this appears to be a useful area in the mechanism of transport molecules being addressed using all cellular systems. These vesicle-mediated transport proteins are now being targeted, or manipulated, for improving cell biocompatibility, such as the synthesis of proteins to improve binding site efficiency, or the use of antibodies to improve the specificity of the delivery system administration of drugs. Other approaches include the use of cell surface receptors and surface systems, or by including substances that act as inhibitors of vesicle transport. By identifying the internal location of specific transport molecules, directed drug delivery can be used to improve drug biocompatibility. Moreover,

an understanding of the genetic polymorphism of drug-metabolizing enzymes is hoped to enable scientists to better design drugs that are targeted for improved bioavailability within distinct patient populations and to understand the causes for various malabsorption syndromes. It is hoped that by understanding some of the genetic variations seen in the patient population, scientists can better accommodate phenotypic variability observed across clinical practices and treat conditions resulting from defective transport systems.

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## 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 minireview 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-Zahra and Pang, 2000), however, is beyond the scope of this work.

The intestine, in addition to the liver, is an important tissue that regulates the extent of absorption of orally administered drugs, since the intestine and liver are involved in first-pass removal (Gibaldi 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 areas due to the highest concentration of villi and microvilli in these regions, and surface area is least for the ileum (Mages and Dailey, 1986).

The circulation of the intestine is unique in that the intestine is the anterior or portal tissue 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 organs, the liver and the lung, for removal (Gugler et al., 1975; Xu et al., 1989; Hkayama et al., 1990). The concentration of drug entering the intestine (Xu et al., 1989; Hkayama 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; Kimura and Higuchi, 2002).

### Drug Properties

Many efforts exist to interpret 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 (Hogben et al., 1957, 1959; Schanker et al., 1957a,b). Deviations from the predictions were found, and these had been explained by the presence of an unstirred water layer (USWL) (Ruzicki 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 un-ionized (Dressman et al., 1985; Meacham and Symillides, 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 (Ugell 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 (Norinder et al., 1999; Zhao et al., 2001, 2002; Klopman et al., 2002) have been developed to forecast the drug permeation potential. 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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<sup>1</sup> Abbreviations used are: GIT, gastrointestinal tract; USWL, unstirred water layer; P-gp, 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; Mdr1, multidrug resistance-associated protein 1; Caco2, organic anion-transporting polypeptide 2; UGT, UDP-glucuronosyltransferase; P-gp, multidrug resistance; GST, glutathione S-transferase; MRT, mean residence time; P450, cytochrome P450; TM, traditional model; SFM, segregated flow model; STM, segmental, traditional model; SSFM, segmental segregated flow model.

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acceptors, the  $\log P$  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 (Elliott 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 nonelectrolyte 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 (Winkler et al., 1998), and is related to the permeability in the aqueous unstirred water layer ( $P_m$ ) and the permeability across the membrane ( $P_{mem}$ ), and may be estimated from the following equation (Ho et al., 1983).

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

The fraction of dose absorbed ( $F_a$  or  $C_{in,system}/C_{in,lumen}$ ) 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 (Fai et al., 1994; Sadee et al., 1995; Truji and Tsumi, 1996; Arimori and Nakano, 1998; Cuddock et al., 1998; Koepsell 1998; Ito 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) (Roensch et al., 1975; Watkins et al., 1987; Dubey and Singh, 1988a; Kohari et al., 1992; Thummet 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; Suzuki 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)] (Talebault et al., 1987; Haing et al., 1992; Smit et al., 1998a,b) and multidrug resistance-associated protein 2 (MRP2) (Paulmann 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 (Saitoh et al., 1996; Lowy et al., 1997b; Kim et al., 1998). Many drugs are substrates of both Pgp and cytochrome P450 3A (Teraso et al., 1996; Wachter et al., 1998; Benet and Cummins, 2001). These are exemplified by verapamil (Saitoh and Awogit, 1995; Sandstrom et al., 1998); anticancer drugs such as vincristine, etoposide, daunorubicin, and paclitaxel (Lee and Hoang, 1995; Bonnichsen et al., 1995; Nakayama et al., 2000; Chiao et al., 2001; Wachter et al., 2001; Abraham et al., 2002); digoxin (Cavet et al., 1996; Greiner et al., 1999); indinavir, the human immunodeficiency virus protease inhibitor (Hochman et al., 2000, 2001; Li et al., 2002); and immunosuppressive agents cyclosporin (Gao 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).

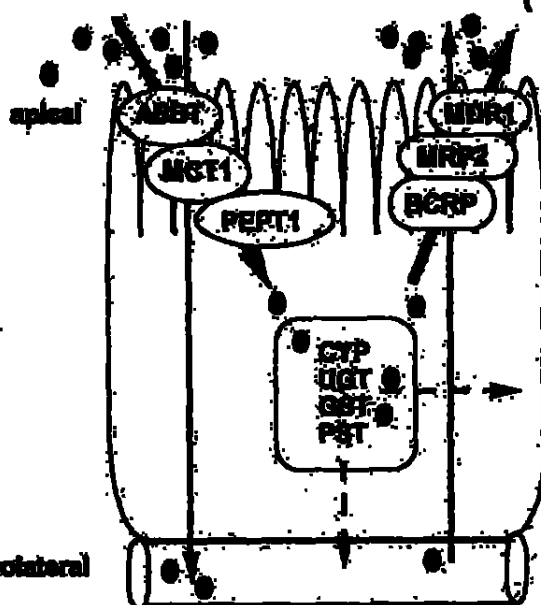


Fig. 1. Schematic diagram of the enterocyte of the intestine showing absorptive and efflux transporters at the apical and basolateral membranes, and enzymes for intracellular metabolism.

#### 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 (Wellington, 1984). Double peak absorption has been correlated with antral gastric motility (Oberle and Amidon, 1987; Pinquello et al., 1987; Saito et al., 1992; Langguth et al., 1994; Lipka et al., 1995; Saito 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 (Bask et al., 2002) or bile salts (Lewin and Raghav, 1993). The data showing double peaks during absorption have been modeled as the discontinuous oral absorption model (Witcher and Bondino, 1996). Inevitably, 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 secretion 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                          | ↓                  | ↓                    |

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TABLE 2  
Involvement of intestinal transporters for drug absorption (see also references mentioned in Tugli and Tamai, 1996)

| Transporter                                                         | Substrate                                                                                                                                                                                                                                           | Reference                                    |
|---------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|
| Slc10 or Slc11<br>Ileal sodium-dependent bile acid transporter      | Thiosulphate                                                                                                                                                                                                                                        | Schneider et al. 1993; Crockett et al., 1998 |
| Pep1<br>Oligopeptide transporter 1                                  | Aminoglycosides, $\beta$ -lactam lactams<br>acetic acid (erythromycin, cephalosporins),<br>acetylsalicylic acid (aspirin) (benzocaine,<br>quinolones), benzoin, rosin, salicylic acid<br>Lactate and pyruvate transport (simple and<br>alkyl acids) | Fel et al., 1994                             |
| Mct1<br>Monocarboxylic acid transporter 1                           | Thiosulphate, oxoanion                                                                                                                                                                                                                              | Waters et al., 2000                          |
| Oatp3<br>Intestinal organic anion transporting<br>polypeptide 3     | Paracetamol, fentanyl                                                                                                                                                                                                                               | Tugli and Tamai, 1998                        |
| NPT1<br>Sodium-dependent phosphate<br>transporter 1                 | Spermidine, spermidine                                                                                                                                                                                                                              |                                              |
| OCT1<br>Organic cation transporter 1                                | Metformin                                                                                                                                                                                                                                           |                                              |
| Ple1<br>Ple1 acid transporter                                       | Zidovudine, stavudine, dymedine, ribavirin,<br>allopurinol, 5-fluorouracil                                                                                                                                                                          |                                              |
| CNT<br>Nucleoside transporters N1 (purine)<br>and N2 (pyrimidine)   | D- and L-methionine, L-methionine, baclofen,<br>tacrine, thiamine                                                                                                                                                                                   |                                              |
| Amino acid carrier                                                  | Desferrioxamine, rhodamine 123, verapamil,<br>diltiazem, colchicine, digoxin, quinine,<br>vincristine, atropine $\beta$ -blockers (atenolol,<br>pindolol, celiprolol, talinolol)                                                                    |                                              |
| MDR1 or Pgp<br>Multidrug resistance protein 1 or P-<br>glycoprotein | Cholesterol esters, glucuronide conjugates,<br>lactoferrin CA, vincristine                                                                                                                                                                          | Gotsh et al., 2000; Motono et al., 2000      |
| MRP2<br>Multidrug resistance-associated<br>protein 2                | Topotecan, irinotecan, irinotecan                                                                                                                                                                                                                   | Dayle et al., 1998; Zamber et al., 2003      |
| BCRP<br>Breast cancer resistance protein                            |                                                                                                                                                                                                                                                     | Rost et al., 2002                            |
| MRP3                                                                |                                                                                                                                                                                                                                                     |                                              |

1) acid-labile compounds, 1) relatively insoluble compounds, 3) drugs with good water and lipid solubility, and 4) drugs absorbed by carriers. For the acid-labile drugs such as penicillin and ampicillin, 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 sound 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 denaturation 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., 1998b; Cvetkovic et al., 1999; Gotsh 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; Lesker and Rickert, 1978; Johnson et al., 2001), cells (Koster and Noordhock, 1983; Traber et al., 1991), everted sacs (Munck, 1965; Barr and Riegelman, 1970; Kaplan and Coffin, 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 absorption has been studied with vesicles prepared from the basolateral and apical membranes (Weinberg 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 (Burton et al., 1993; Hosoya et al., 1996; Tomika et al., 1996; Teruo 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 $\alpha$ -25-dihydroxy vitamin D<sub>3</sub> for 2 weeks postconfluence, cytochrome P450 3A activities were up-regulated (Crepsi et al., 1996, 2000; Schmedlun-Ben et al., 1997; Thummel et al., 2001). More recent development involved transfection with the cytochrome P450 gene and stimulation by butyrate (Cammins 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.

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TABLE 3

Segmental distribution of transporters and enzymes among the intestinal segments

| Transporter       | Segmental Distribution                                                     | References                                                    |
|-------------------|----------------------------------------------------------------------------|---------------------------------------------------------------|
| Abs1              | distal intestine > proximal intestine                                      | Schneider et al., 1993; Akhtz et al., 1996                    |
| Pgp1              | proximal intestine > distal (bow function)<br>(jejunum > ileum > duodenum) | Pol et al., 1994                                              |
| Mct1              | duodenum < jejunum > ileum                                                 | Tamari et al., 1999; Cong et al., 2001                        |
| Oatp3             | highest in jejunum                                                         | Waters et al., 2000                                           |
| CNT               | highest in jejunum                                                         | Ngo et al., 2001                                              |
| CYP3A             | duodenum ~ jejunum > ileum                                                 | Paine et al., 1996, 1997; Li et al., 2002                     |
| UGT               | duodenum ~ jejunum > ileum                                                 | Kozar et al., 1983                                            |
| PST               | duodenum ~ jejunum > ileum                                                 | Schwartz and Schwach, 1974                                    |
| GST               | duodenum ~ jejunum > ileum                                                 | Pliska et al., 1977                                           |
| Biotransformation | proximal > distal                                                          | Hofigebauer et al., 1984                                      |
| Mrp2              | duodenum ~ jejunum > ileum                                                 | Motilke et al., 2000; Gorch et al., 2000                      |
| Mdr1              | jejunum ~ ileum > duodenum                                                 | Belich and Amagat, 1992; Collet et al., 1999; Li et al., 2002 |
| Mdr3 (bilecanal)  | ileum > jejunum > duodenum                                                 | Kost et al., 2002                                             |

TABLE 4

Route-dependent intestinal metabolism

| Drugs           | Route of Administration           |          | References                               |
|-----------------|-----------------------------------|----------|------------------------------------------|
|                 | Intestine                         | Systemic |                                          |
| Acetaminophen   | Glucuronidation                   | NO       | Pang et al., 1986                        |
| Erythromycin    | Erythromycin                      | NO       | Pang et al., 1985                        |
| Cyclosporin     | +                                 | NO       | Dunne et al., 1985                       |
| Tacrolimus      | +                                 | NO       | Lampson et al., 1995                     |
| Midazolam       | 1-Hydroxymidazolam                | low      | Paine et al., 1996; Thummet et al., 1996 |
| (-)-Amphetamine | (-)-Carbovir                      | low      | Wan et al., 1999                         |
| Morphine        | Morphine 3 $\beta$ -O-glucuronide | NO       | 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 (Windmueller and Spaeth, 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 Dobut method entails use of an *in situ* rat gut technique for drug administration into the lumen (Dobut et al., 1969; Sim and Back, 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 Ringelman, 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 (Grammett and Richter, 1994; Grammett, 1996; Grammett et al., 1994, 1996). Gene knockout mice (Kim et al., 1998; Smit et al., 1998; Greiner et al., 1999) and mutant animal models (Gotoh 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; Effeney 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 (Paine 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, 1983, 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 Tamari, 1996; Knepsell, 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 midazolam (Unger et al., 1996), nifedipine (Racof et al., 1998), acetaminophen (Grammett and Richter, 1994), griseofulvin (Grammett, 1996), and (-)-carbovir (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 nifedipine (Bottle and Broutner, 1995) and diltiazem (Hosny et al., 1995). In like fashion, the absorption of nifedipine

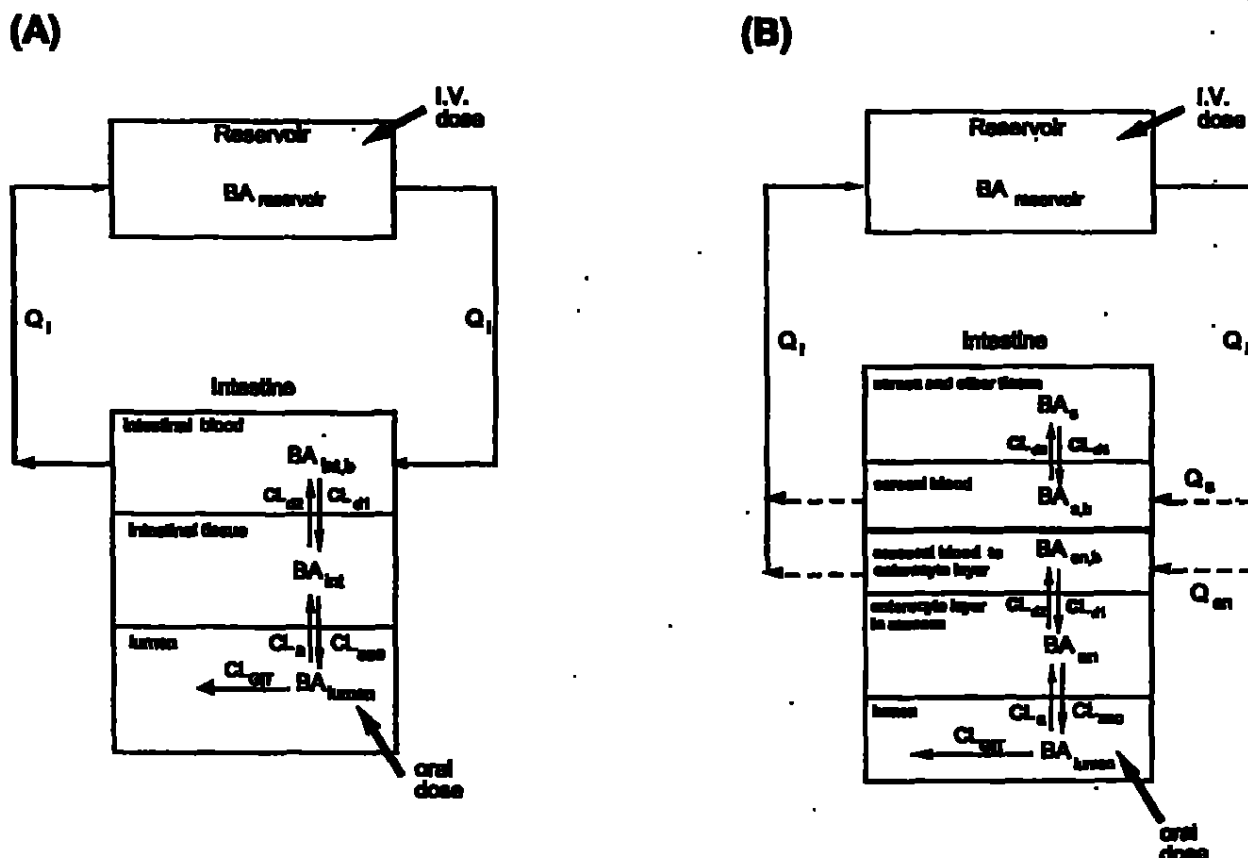


FIG. 2. Fate of drug and metabolite in the TM (A) and IFM (B).

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 ( $Q_i$ ) perfuses the entire intestinal tissue, the site of metabolism and absorption from the lumen. For IFM, intestinal blood is segregated to perfuse the nonmetabolizing ( $Q_i$ ) and enterocyte-mucosal ( $Q_{en}$ ) regions. Drug equilibria with these in the corresponding tissue layers with intrinsic transfer clearances  $CL_{int}$  and  $CL_{en}$  for TM or  $CL_{sb}$  and  $CL_{en}$  and  $CL_{en}$  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 mucosal intrinsic clearances,  $CL_{int}$  and  $CL_{en}$ , respectively. Gastrointestinal transit clearance is denoted by  $CL_{GIT}$ . Adapted from Cong et al. (2000), with permission.

(Gramatté et al., 1994) and talinolol (Gramatté et al., 1996) is higher in the proximal intestine in humans. For hydrochlorothiazide, atenolol, furosemide, and cimetidine (Lennernäs, 1998), the net mucosal to serosal absorption was greater for the jejunum than for the ileum. For verapamil (Hunter et al., 1990), phenytoin, alendronate, gemfibrozil, metoprolol, omeprazole, propranolol, flunarizine, erythritol, dDAP (Ugall et al., 1998), and etoposide (Mahbey 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; Motino 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 Mdr1 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 rat 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). Gotoh et al. (2000) demonstrated the greater mRNA expression of Mdr2 in the rat jejunum, followed by the duodenum and ileum, with very little in the colon, as confirmed by Motino et al. (2000). The expression of the glutathione conjugate 2,4-dinitrophenyl-S-glutathione by Mdr2 was greatest in the jejunum, as expected by mRNA expression (Gotoh et al., 2000). The efflux transporter rat Mdr2 is higher in the proximal region (Gotoh et al., 2000; Motino 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; Chénale et al., 1995; Lowy et al., 1997b; Collett et al., 1999; Nakayama et al., 2000; Stephens et al., 2001; Li et al., 2002). The rat basolateral Mdr3 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 (STs), and glutathione S-transferases (GSTs) exhibit a decreasing gradient along the

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intestinal wall, from duodenum to ileum (Clifton and Kaplowitz, 1977; Pinkas et al., 1977; Schwarz and Schwark, 1984; Doherty 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 (Hosoe 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), enalapril (Pang et al., 1985) morphine (Doherty and Pang, 2000) and (-)-aminocaproic, 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 ashepatic 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 (Lown et al., 1994, 1997b) that is ordinarily used to correlate with liver CYP3A4 levels (Watkins et al., 1989). The exclusivity 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 prescriptive 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 prescriptive 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 (Gruener 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 least 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 (Tan 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 (Tan 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

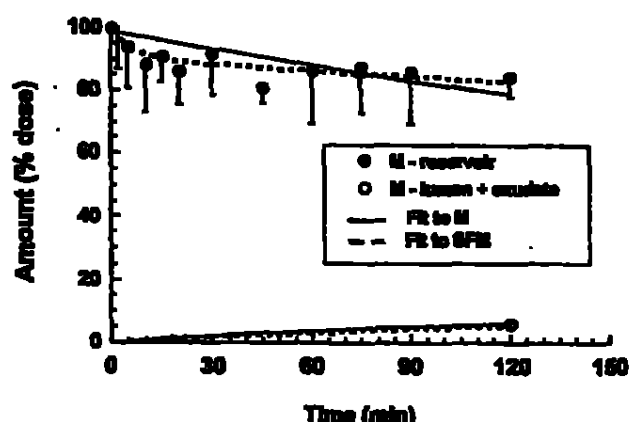


FIG. 3. Fit of tracer  $[^3\text{H}]$ morphine data from the vascularly perfused, recirculating rat small intestine preparation to the TM and SPM, after systemic administration of dose into the reservoir (data from Cong et al., 2000).

There was a total lack of morphine glucuronide formed. Only morphine was excreted into the lumen (and luminal fluid that was collected as excreted). Note the superior fit of the data to the SPM. Adapted from Cong et al. (2000), with permission.

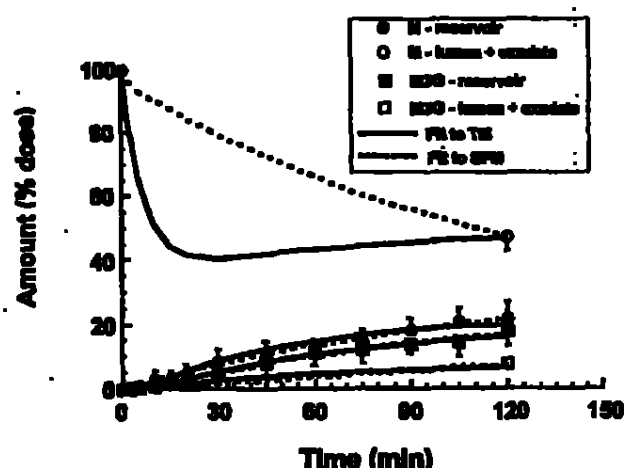


FIG. 4. Fit of tracer  $[^3\text{H}]$ morphine data from the vascularly perfused, recirculating rat small intestine preparation to the TM and SPM, after luminal administration of dose into the intestinal lumen (data from Cong et al., 2000).

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 excreted. Note the superior fit of the data to the SPM. Adapted from Cong et al. (2000), with permission.

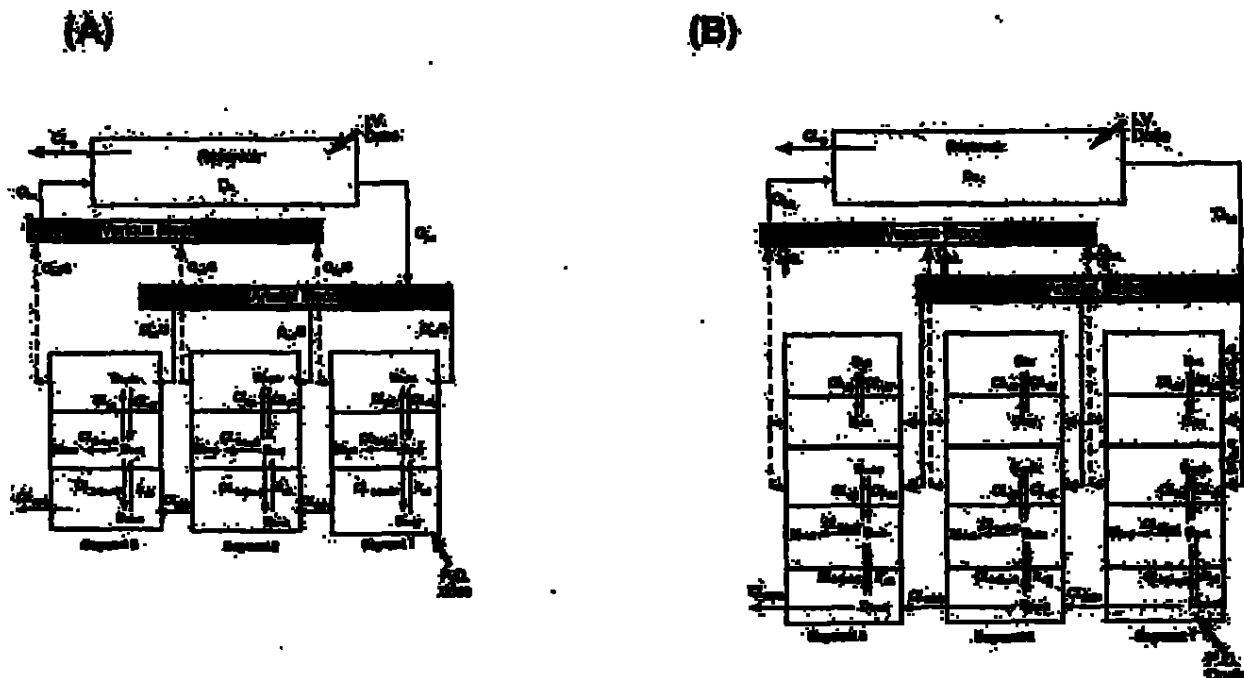


Fig. 5. The SFM, which views the intestine as three equal segments (or expanded  $l^*$  compartments where  $l = 1, 2$ , or  $3$ ) of TM (Fig. 2A) with metabolism taking place to form the metabolite (M) in the intestinal tissue (A), and the SFM, an expansion of the SFM (Fig. 2B), which views the intestine as three segments (or expanded  $l^*$  compartments where  $l = 1, 2$ , or  $3$ ) with low and partial flow to the enterocyte regions ( $f_e Q_{int,A}$ ), where  $f_e$  is the fraction of total intestinal flow ( $Q_{int}$ ) perfusing the enterocyte region, where the enzymes and efflux transporters reside and bulk flow  $[(1 - f_e) Q_{int,A}]$  to the "other" nonabsorbing region (B).

Drug (D) in blood equilibrates with that in the corresponding tissue layers with intrinsic transfer clearances  $CL_{int}$  and  $CL_{ext}$  for both SFM and SSFM. 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 intrinsic clearances,  $CL_{met}$  and  $CL_{sec}$ , respectively, for each  $l^*$  segment. The gastrointestinal transit clearance is denoted by  $CL_{GIT}$ . Adapted from Tian et al. (2003b), with permission.

varying transit times and absorption (Kimura 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 human, 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; Lowa et al., 1997a; Dresser et al., 2002).

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

based models that encompasses 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 redistribution of tracer morphine in the perfused rat small intestine preparation (Doherty and Pang, 2000). In this prepa-

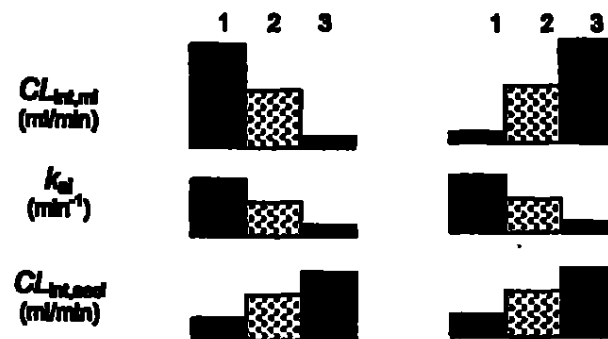


Fig. 6. Simulations based on intestinal modeling for the rat small intestine, to determine the varying distributions of absorptive and secretory transporters and metabolic enzymes that evoke high and low bioavailabilities.

Segmental distributions (designated as  $l^*$  segments where  $l = 1, 2$ , and  $3$ ) of the metabolic intrinsic clearance,  $CL_{met}$  (0.5, 0.5, and 0.1 ml/min), the absorption rate constant,  $k_a$  (1, 3, and 1 min<sup>-1</sup>), and the secretory intrinsic clearance,  $CL_{sec}$  (2, 4, and 6 ml/min) simulated the lowest bioavailability (P) (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 0.5 ml/min, and  $k_a$  values were 5, 3, and 1 min<sup>-1</sup>) according to the predictions of the SFM and SSFM. The predicted difference existed in the descending vena according to the predictions of the SFM and SSFM. Taken from Tian et al. (2003b), with permission.

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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 Noordhoek (1985) (Fig. 2B), was developed. SFM 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 SFM recognizes the subtle demarcation of these layers and distributions in blood supply (Svanvik, 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% (Mieszkier et al., 1976; MacFarran and Malins, 1977; Malins 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 splanchnic regions. The condition conduces to a greater intestinal metabolism with oral versus intravenous dosing. For data on tracer morphine glucuronidation in the recirculating, vascular perfused rat small intestine, the SFM 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 MRT. Under linear conditions, intestinal secretion results in reduced intestinal metabolite formation, as confirmed in a recent theoretical examination of TM and SFM (unpublished data of K. S. Pang, R. Tsang, and D. Tam). The con-

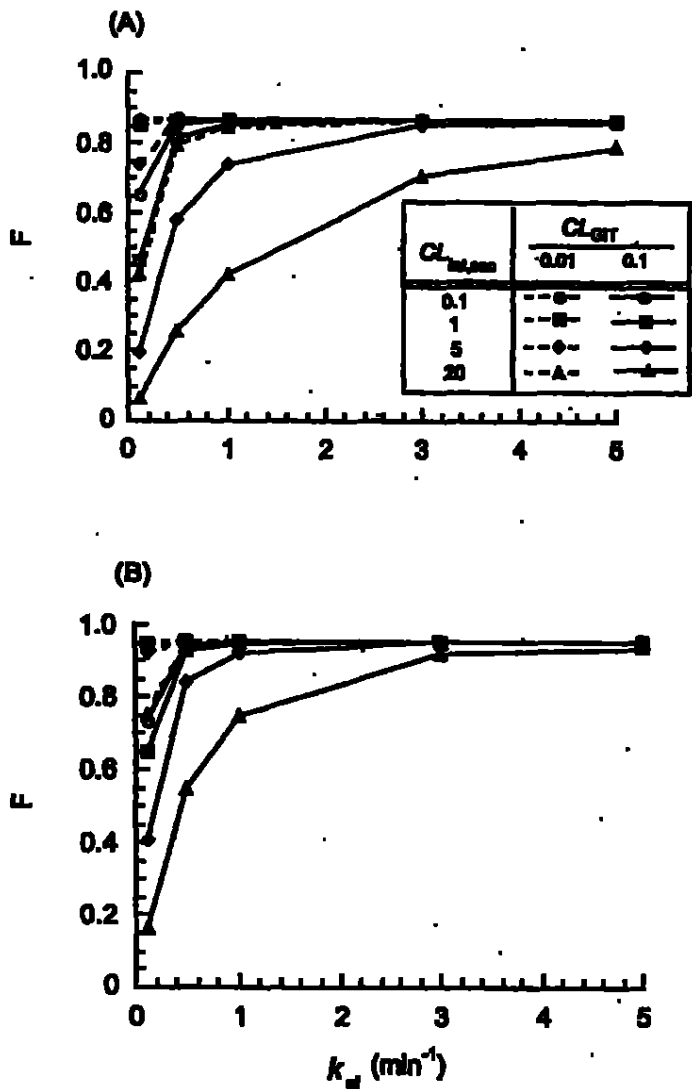


FIG. 7. Simulations performed for the SFM (A) and SFM (B) to demonstrate the effect of the segmented, gastrointestinal transit clearance ( $CL_{GIT} = 0.01$  or  $0.1 \text{ ml/min}$ ), the secretory intrinsic clearance ( $CL_{int,sec}$ ), and the absorption rate constant ( $k_a$ ) on the intestinal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

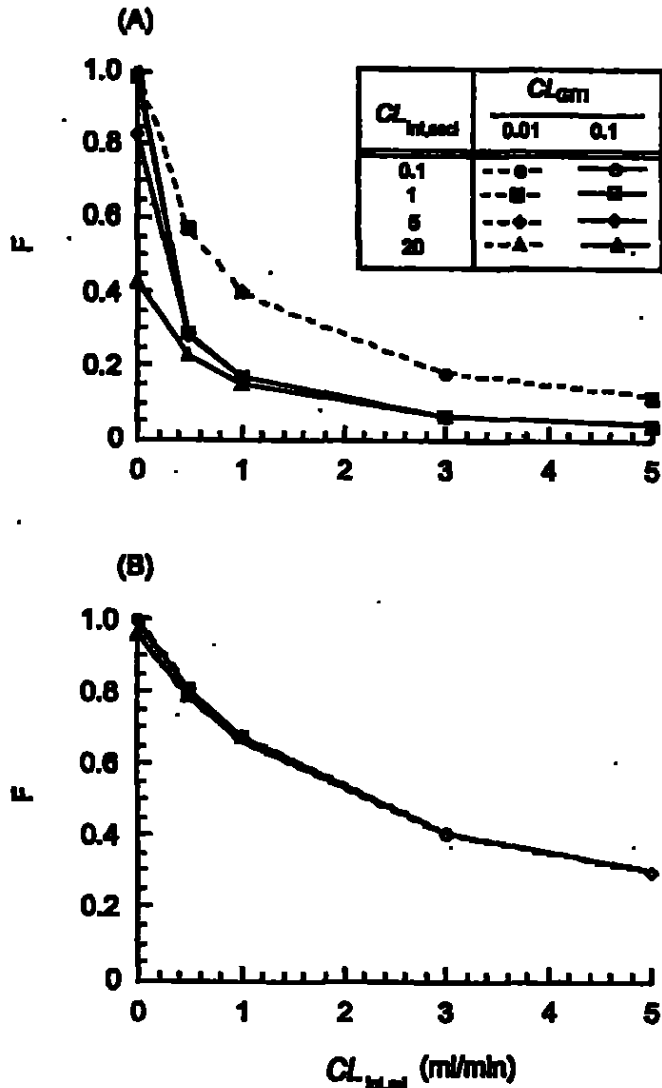


FIG. 6. Simulations performed for the STIM (A) and SSFM (B) to demonstrate the effect of the gastrointestinal tract clearance ( $CL_{int,m} = 0.01$  or  $0.1$  ml/min), the secretory intestinal clearance ( $CL_{int,sec}$ ), and the metabolic intestinal clearance ( $CL_{int,m}$ ) on the intestinal (systemic) bioavailability,  $F$ .

The absorption rate constant for the each segment,  $k_a$ , was kept at  $3 \text{ min}^{-1}$ ; the partitioning clearance for drugs,  $CL_{int}$  and  $CL_{int,p}$ , was  $0.9 \text{ ml/min}$ . 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.

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 (Sikroni and Pang, 1997; Schuetz 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 reduce the bioavailability (Table 1).

**Segmental, Traditional Model (STIM) 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 (Abo-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 STIM 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 fast 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) (Tam 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 mucus 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/secretion, 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 SFM and SSFM, 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 (Ichimaki et al., 1997). The disparate 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 (Glaeser 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 (Tamai et al., 1997; Tam 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-5RW), 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-5RW 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 administered 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-5RW provides a promising way to increase the dissolution rate and the oral absorption of poorly water-soluble drugs such as ER-34122. © 2001 Wiley-Liss, Inc. and the American Pharmaceutical Association *J Pharm Sci* 91:258–266, 2002

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

## INTRODUCTION

When a poorly water-soluble drug is orally administered, 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<sup>1–3</sup> have been investigated to increase the dissolution rate. One of the approaches is the formation of solid dispersions with pharmacologically inert, polymeric materials.<sup>4–7</sup> Many solid dispersion techniques have been employed to enhance the dissolution rates of poorly water-soluble drugs, such as digoxin,<sup>8</sup> ethenzamide,<sup>9</sup> nifedipine,<sup>10–12</sup> furosemide,<sup>13,14</sup> griseofulvin,<sup>15,16</sup> and tolbutamide.<sup>17</sup>

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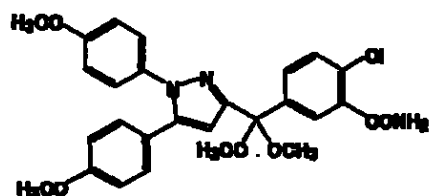


Figure 1. Molecular structure of ER-34122.

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

ER-34122 (Fig. 1), a potent dual 5-lipoxygenase/cyclooxygenase inhibitor with anti-inflammatory activity,<sup>19,20</sup> 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  $4.948 \pm 1.621$  and  $0.029 \pm 0.012$   $\mu\text{g} \cdot \text{h/mL}$ . 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<sup>®</sup>, hydroxypropylmethylcellulose, methylcellulose; methylcellulose (MC); and HP-55S<sup>®</sup>, hydroxypropylmethylcellulose phthalate were purchased from Shinetsu Chemical Industry Company, Ltd. DK-SS<sup>®</sup> and P-1570<sup>®</sup>, 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<sup>®</sup>, 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<sup>®</sup>, 0.45  $\mu\text{m}$ , Nihon Millipore, Ltd., Yonemawa, 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<br>( $\mu\text{g/mL}$ ) |
|-------------------------|--------|------------------------------------|
| Species                 | pH     |                                    |
| Britton Robinson buffer | pH 2   | $\leq 0.01$                        |
| Britton Robinson buffer | pH 3   | $\leq 0.01$                        |
| Britton Robinson buffer | pH 4   | $\leq 0.01$                        |
| Britton Robinson buffer | pH 5   | $\leq 0.01$                        |
| Britton Robinson buffer | pH 6   | $\leq 0.01$                        |
| Britton Robinson buffer | pH 8   | $\leq 0.01$                        |
| JP 1 medium             | pH 1.2 | $\leq 0.01$                        |
| JP 2 medium             | pH 6.8 | 0.03                               |

prepared by the solvent evaporation technique, wherein 6 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-5HW 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-5HW 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  $\mu$ 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 $\alpha$  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  $2\theta$  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  $37 \pm 0.5^\circ\text{C}$  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-303 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-620 spectrometer (Jasco, Tokyo, Japan). Samples were scanned as potassium bromide pellets with an instrument resolution of 4  $\text{cm}^{-1}$ . Data were collected over the spectral region from 4000 to 400  $\text{cm}^{-1}$ .

#### Hygroscopicity Measurements

Vapor pressure isotherm data for ER-34122 alone and the 1:1 (w/w) drug/TC-5HW 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 3 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-5HW dispersion were carried out using male beagle dogs ( $n = 8$ ). 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, 8, 8, and 24 h post administration. The blood samples were immediately centrifuged, and the plasma samples were stored at  $-20^\circ\text{C}$  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  $\mu\text{g/mL}$  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.

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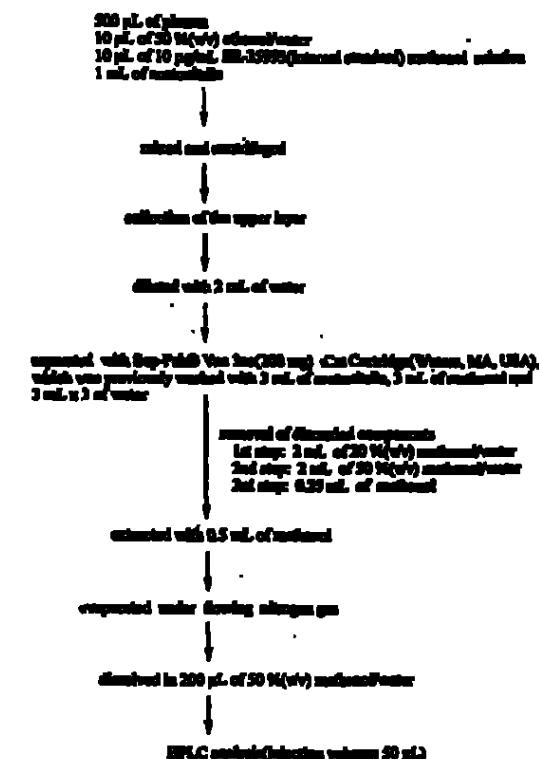


Chart 1. Pretreatment flow of plasma sample for HPLC analysis of ER-34122.

## 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-34122 from these solid dispersions, which showed no diffraction peaks in the XRD patterns, were significantly faster than that from the pure drug,<sup>14,21-23</sup> 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-34122 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

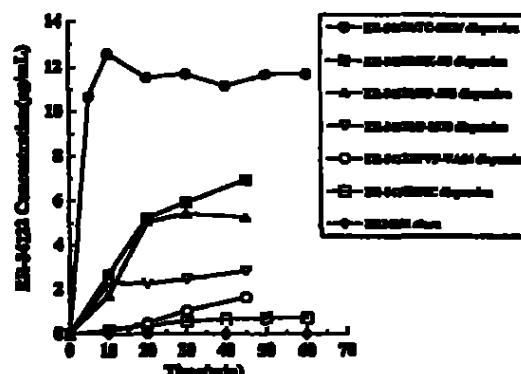


Figure 2. Dissolution profiles of ER-34122 from 1:5 (w/w) drug/carrier dispersions in JP 2 medium at 37°C. Six kinds of water-soluble polymers (i.e., TC-5RW, DE-88, HP-558, P-1570, PVF-VA64, and MC) were used for the preparation of 1:5 (w/w) drug/carrier dispersions.

be the most suitable for the ER-34122 dosage form.

### Physicochemical Property

The XRD patterns for ER-34122 alone, the 1:1 (w/w) drug/TC-5RW dispersion, and the corresponding physical mixture are shown in Figure 4. ER-34122 is crystalline, as demonstrated by its sharp and intense diffraction peaks, whereas the solid dispersion showed no diffraction peaks, suggesting that ER-34122 was in the amorphous state.<sup>7</sup> In contrast, the physical mixture showed several diffraction peaks attributable to the crystalline form of the drug.

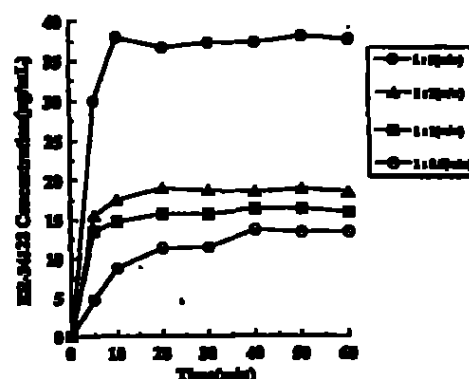


Figure 3. Effect of TC-5RW content on the release of ER-34122 from solid dispersions. Drug/TC-5RW dispersions with four levels of TC-5RW content [1:0.5, 1:1, 1:2, and 1:5 (w/w)] were studied.

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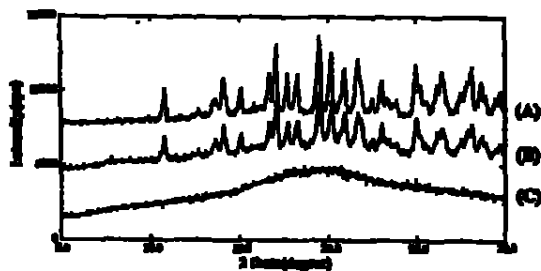


Figure 4. XRD patterns of ER-34122 alone, 1:1 (w/w) ER-34122/TC-5RW dispersion, and the corresponding physical mixture: (A) ER-34122 alone, (B) the physical mixture, and (C) the solid dispersion.

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  $2000\text{ cm}^{-1}$ , no differences were 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.<sup>24-26</sup> From the FTIR spectra of ER-34122, an absorption band was observed at  $1667\text{ cm}^{-1}$ , which is assigned to the C=O bond stretching vibration of  $\text{Ar-CONH}_2$ .<sup>27</sup> The absorption band was upshifted to  $1684\text{ cm}^{-1}$  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,<sup>10,11</sup> 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.<sup>28</sup>

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  $37^\circ\text{C}$  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  $37^\circ\text{C}$

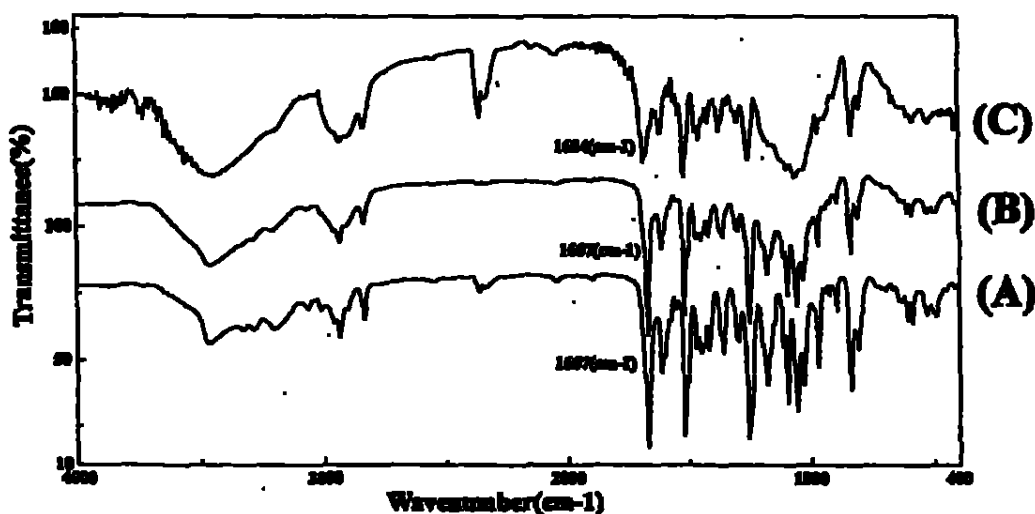


Figure 5. FTIR spectra of ER-34122 alone, 1:1 (w/w) ER-34122/TC-5RW dispersion, and the corresponding physical mixture from  $4000\text{ to }400\text{ cm}^{-1}$ : (A) ER-34122 alone, (B) the physical mixture, and (C) the solid dispersion.

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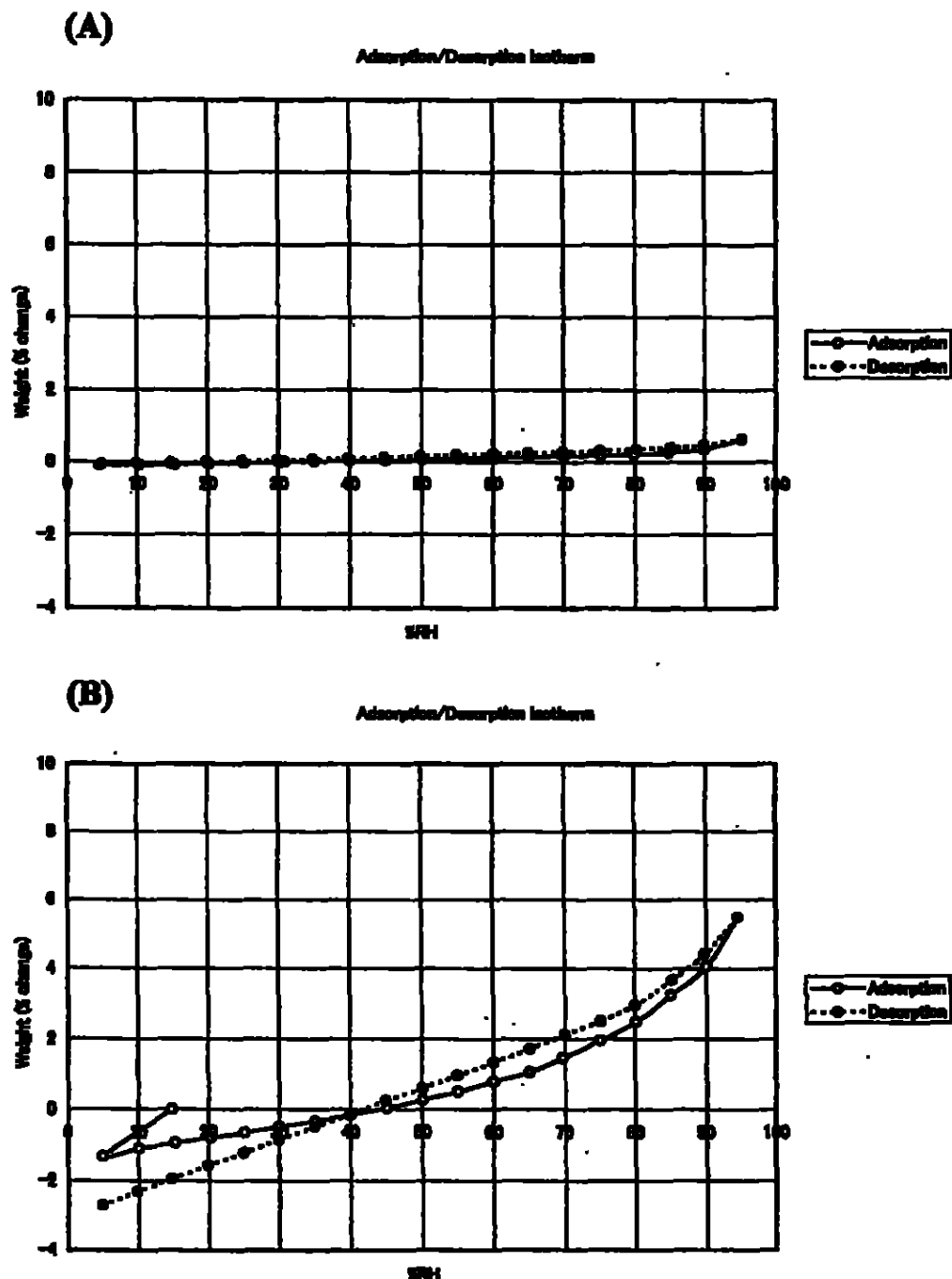


Figure 6. Adsorption-desorption isotherm for ER-34123 alone and 1:1 (w/w) ER-34123/TC-5RW dispersion: (A) ER-34123 alone and (B) the solid dispersion.

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

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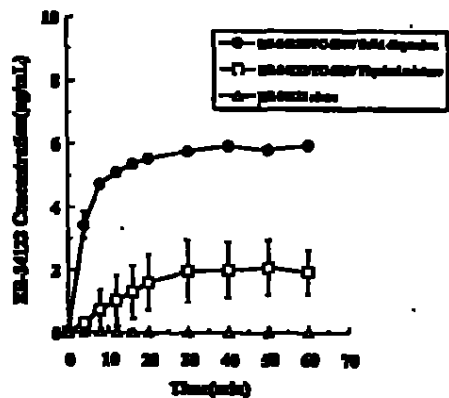


Figure 7. Dissolution profiles of ER-34122 alone, 1:1 (w/w) ER-34122/TC-5RW dispersion, and the corresponding physical mixture in JP 2 medium at 37°C. The test was carried out in triplicate.

precipitation of ER-34122 or lowers the surface tension of the medium,<sup>20</sup> resulting in better wettability.<sup>2,7,20</sup> 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.<sup>21–23</sup> 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.

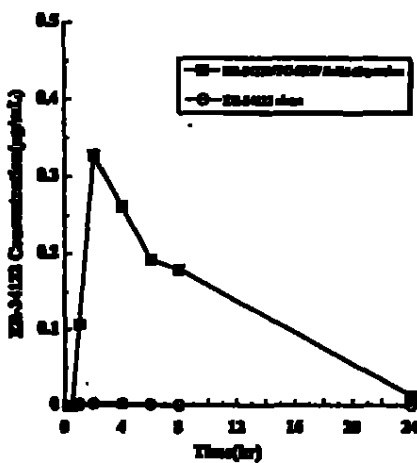


Figure 8. Plasma levels of ER-34122 after the pure drug and 1:1 (w/w) drug/TC-5RW dispersion were orally administrated at the dose of 10 mg/kg as ER-34122 to beagle dogs ( $n = 3$ ).

**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.<sup>24,25</sup> 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 ( $n = 3$ )<sup>a</sup>

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

<sup>a</sup>Each 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 carbamazepine<sup>1</sup>

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Differences in the bioavailability after administration of two pure carbamazepine samples that were different in particle size 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. (Finlepin®)

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].

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

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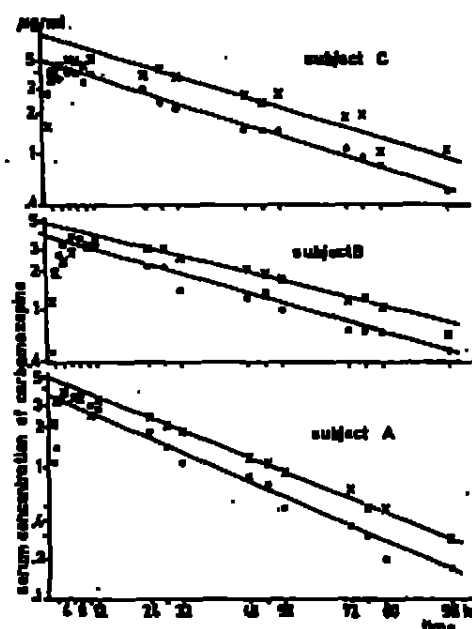


Figure 1. Plasma concentrations of carbamazepine in 3 healthy volunteers following oral administration of 400 mg each of carbamazepine 1 and 2.

| 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

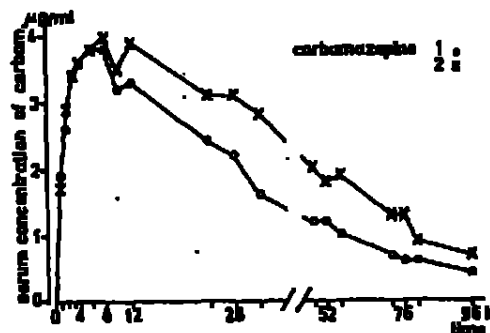


Figure 2. Mean values of the plasma carbamazepine levels of sample 1 and sample 2 in 3 volunteers following oral administration of 400 mg.

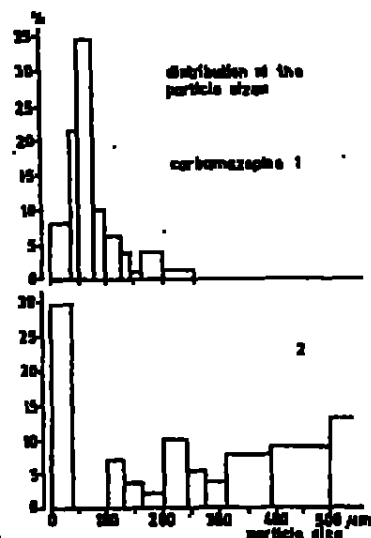


Figure 3. Distribution of particle sizes of carbamazepine 1 and carbamazepine 2.

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  $\mu\text{m}$ , lot A, 3.2  $\mu\text{m}$ , lot B, 12.0  $\mu\text{m}$ , 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  $\mu\text{m}$ ) was significantly ( $P < 0.05$ ) less-bioavailable than was suspension A (dw = 1.65  $\mu\text{m}$ ), 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 (80% particles < 10  $\mu\text{m}$ ) 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 horses, 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.<sup>1,2</sup> The drug has a relatively low aqueous solubility of less than 4  $\mu\text{g/ml}$  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.<sup>3,4</sup> Particle size distribution is a major factor in the efficiency of phenothiazine and some other benzimidazole derivatives as anthelmintics in ruminants.<sup>4-6</sup>

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, Mroczek, Parekh, Nguyen, Schiltz), and Commonwealth Scientific and Industrial Organization, Division of Animal Health, McMaster Laboratories, Sydney Australia (Prichard, Hannaway).  
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Demonstration of efficacy against *Haemonchus contortus* of this benzimidazole-resistant *H. contortus*. Unpublished data, Syntex Research, Palo Alto, Calif.

substances through a macropolymer<sup>4</sup> and by subsequently passing it through a micronizer.<sup>5</sup> The milled material was termed lot B before micronization. Lot C was prepared by using the following technique: 680 g of oxandazole raw material was mixed with 10,000 ml of deionized water containing 5 g of polyoxyethylene-40-monostearate.<sup>6</sup> The suspension was passed through a 20- $\mu$ m nylon fabric screen.<sup>7</sup>

The material passing through the 20- $\mu$ m screen was then passed through a 10- $\mu$ m screen. Oxandazole 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 differentiate 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% oxandazole 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 microscope<sup>8</sup> 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  $\mu$ m in diameter. This information was helpful in selecting the appropriate aperture tubes for Coulter counter runs.

Scanning electron microscope (SEM) analysis<sup>9</sup> 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 oxandazole 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 microscope<sup>10</sup> 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 counter<sup>1</sup> equipped

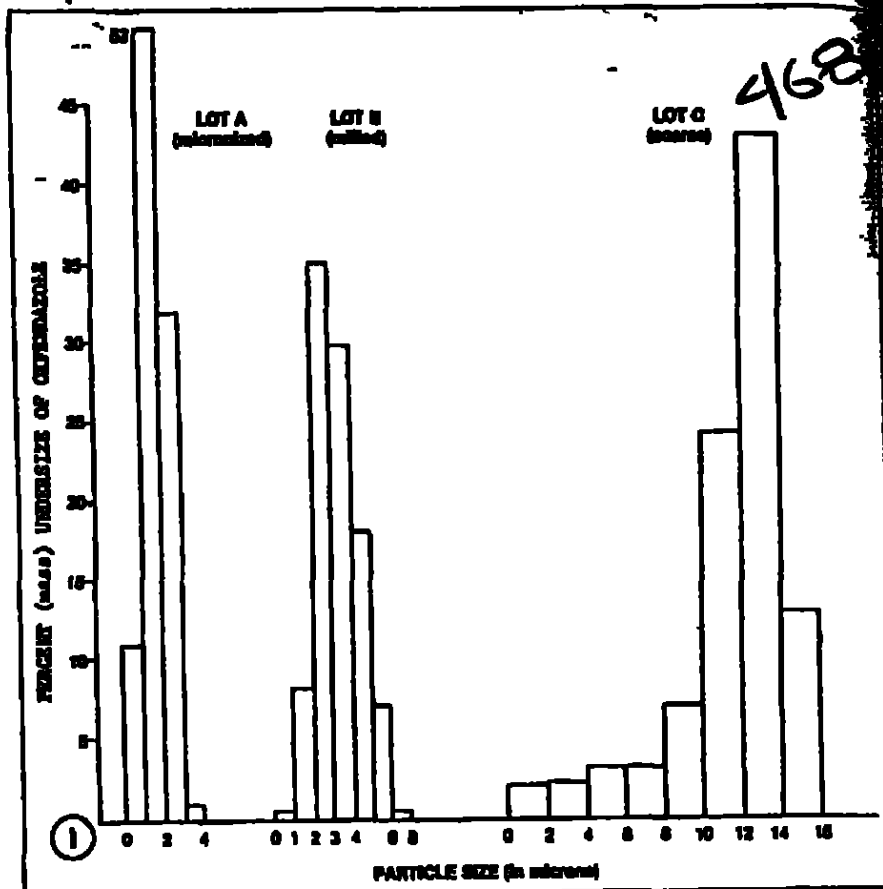


Fig 1—Particle size distribution of oxandazole lots by Coulter counter measurements.

with a triple nanometer and a 50- $\mu$ m, as well as a 100- $\mu$ m aperture tube. The instrument was previously calibrated with 2.02- $\mu$ 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% polyoxyethylene-40-monostearate solution in water that had been previously filtered through a 0.4- $\mu$ m membrane filter.<sup>11</sup> It was adjusted to volume (30 ml) with 0.9% NaCl-ende free balanced electrolyte isotonic solution,<sup>1</sup> 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 30 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 oxandazole 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**—Oxandazole 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.<sup>12</sup> Three independent runs were carried out on each lot of oxandazole. This measurement of specific surface area was based on an adsorption isotherm equation derived by BET. Essentially, the effective surface

<sup>1</sup> Syntex Corp, Palo Alto, Calif.

<sup>2</sup> Millinelli, WJ Martens & Co, Chatham, NJ.

<sup>3</sup> Micron Master, Jet Pulverizer Co, Palmyra, NJ.

<sup>4</sup> KCI America Inc, Wilmington, Del.

<sup>5</sup> TETKO Inc, Elmford, NY.

<sup>6</sup> Bunt Lohs Inc, Wether Germany.

<sup>7</sup> Measurements performed by Surface Sciences Laboratories Inc, Palo Alto, Calif.

<sup>8</sup> Cambridge model 600, Cambridge Instruments, Cambridge, England.

<sup>9</sup> Model TAIL, Coulter Electronics Inc, Elkhart, Fla.

<sup>10</sup> Polysciences 0.45- $\mu$ m filter membrane, Natick, Massachusetts, Calif.

<sup>11</sup> Isoton II, Coulter Diagnostics, Division of Coulter Electronics, Elkhart, Fla.

<sup>12</sup> Quantachrome Corp, Germantown, NY.

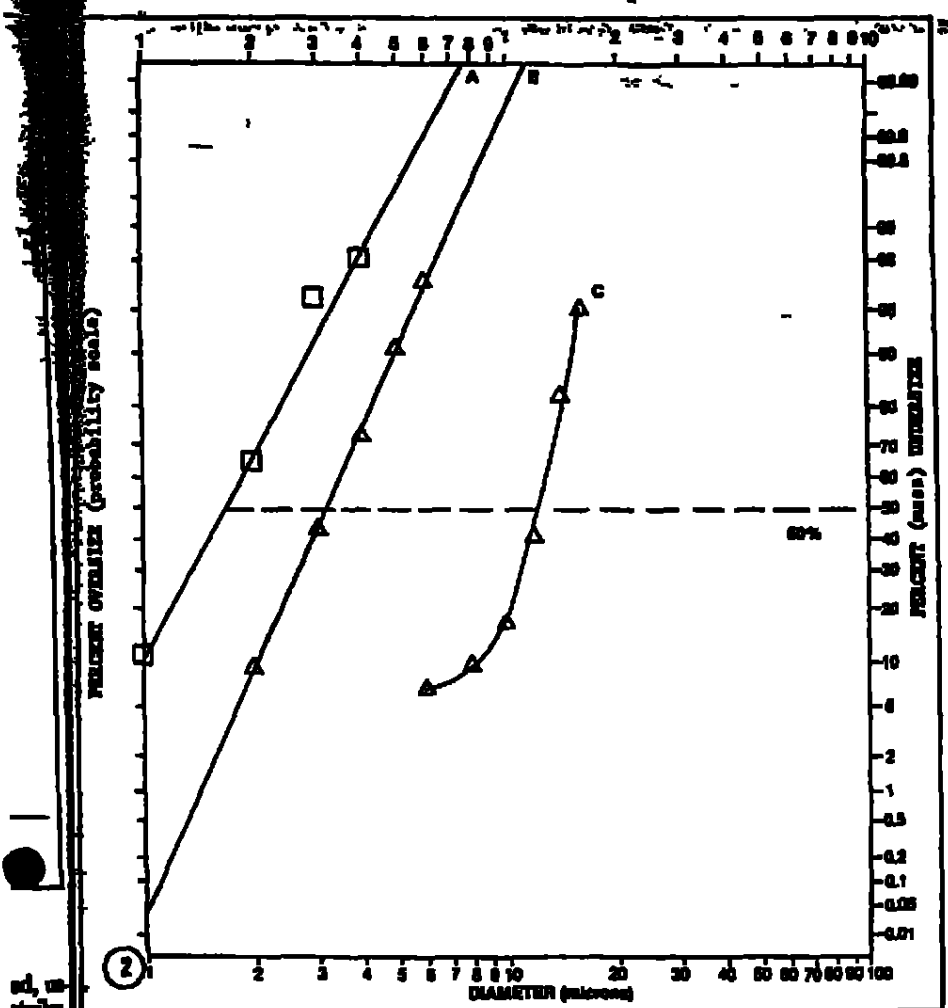


Fig 3—Linear cumulative plot on probit scale representing particle size distribution of oxendazole suspensions A, B, and C.

of powders was determined by measuring the amount of gas adsorbed. Average surface area values and corresponding  $\alpha_n$  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 1.8) acidified with 5 ml of 37.4% HCl containing 0.5% polysorbate 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 22.65 mg of oxendazole, 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 runs. 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). Oxendazole 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  $\alpha_n$  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 3.

**Bioavailability in Sheep**—Twelve normal, young (8 to 12 months old) female Dorset sheep ranging in weight from 38.3 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.) Oxendazole was dosed at a rate of 5 mg/kg of body weight. Suspensions were administered with 10-ml disposable syringes via Tygon stomach tubes (1.27 cm,  $\phi$  × 0.88 cm, in) 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 (base-line 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. Oxendazole was measured by the radioimmunoassay method of Newman et al.<sup>11</sup> Mean oxendazole 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 oxendazole 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

\* Fettec & Biol Inc, Richmond, Va.

\* Pye Unicam Ltd, Cambridge, England.

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

| Suspension | Oxendazole raw material particle size weight mean diameter ( $\mu$ m) | Surface area ( $m^2/g$ ) | Dissolution percentages <sup>a</sup> at various time intervals (min) |                |                | Plasma AUC <sup>b</sup> ( $\mu g/0.1 ml \times hr$ ) |
|------------|-----------------------------------------------------------------------|--------------------------|----------------------------------------------------------------------|----------------|----------------|------------------------------------------------------|
|            |                                                                       |                          | T <sub>1</sub>                                                       | T <sub>2</sub> | T <sub>3</sub> |                                                      |
| A          | 1.86 <sup>c</sup>                                                     | 4.3812 $\pm$ 0.0643      | 91.3 $\pm$ 3.3                                                       | 94.1 $\pm$ 1.3 | 97.3 $\pm$ 0.3 | 2832.00                                              |
| B          | 8.30 <sup>d</sup>                                                     | 2.4895 $\pm$ 0.0649      | 62.5 $\pm$ 7.5                                                       | 81.9 $\pm$ 2.4 | 84.7 $\pm$ 2.6 | 2229.00                                              |
| C          | 12.00 <sup>e</sup>                                                    | 2.1562 $\pm$ 0.0597      | 50.9 $\pm$ 6.5                                                       | 69.3 $\pm$ 6.7 | 78.2 $\pm$ 1.7 | 2121.00                                              |

<sup>a</sup> 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 T<sub>1</sub> and T<sub>2</sub>. <sup>b</sup> At  $P < 0.05$ , with respect to plasma AUC (total area under the plasma concentration vs T curve,  $\mu g/ml \times hr$ ) values, suspension A was significantly different from suspension C. <sup>c</sup> Micronized. <sup>d</sup> Milled. <sup>e</sup> Coarse.

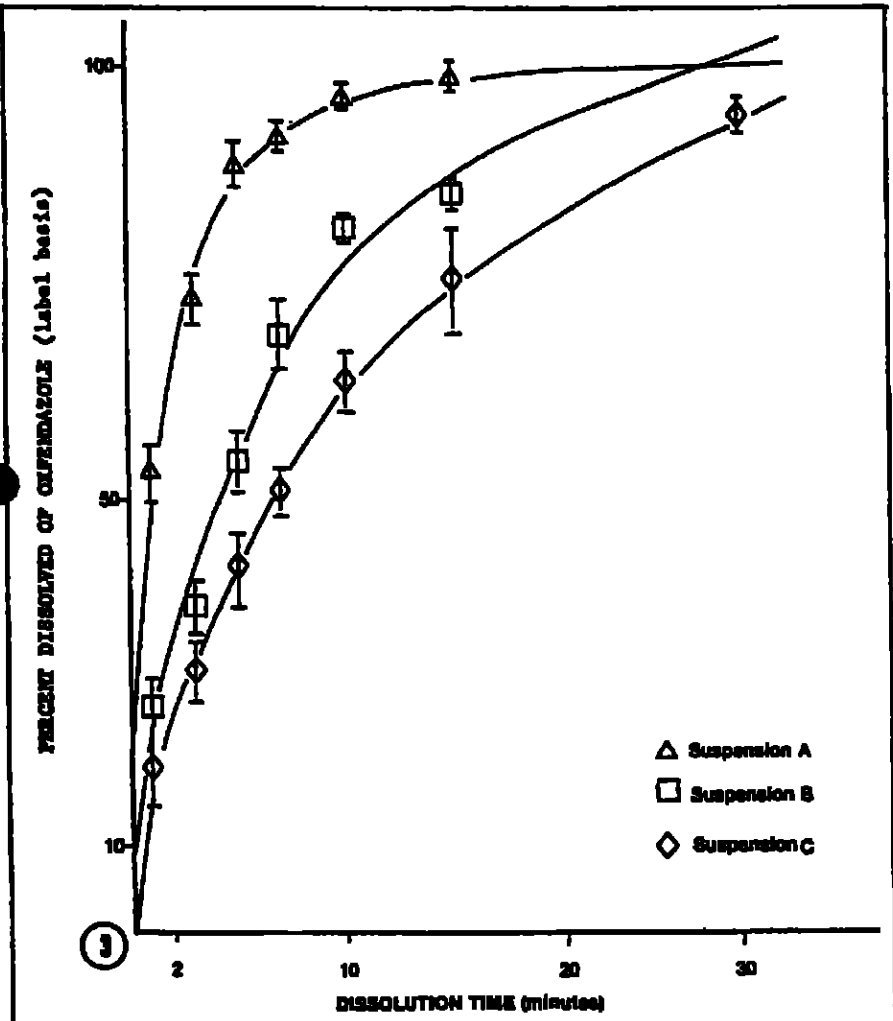


Fig 3—Comparison between dissolution rate of 3 oxendazole suspensions.

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 Merino weaner sheep were infected with 6,000 benzimidazole-resistant *H. contortus* larvae<sup>a</sup> and were maintained in concrete

pens free of adventitious parasite infections. At 28 days after infection, 14 of these sheep were orally drenched with oxendazole suspension at the rate of 5 mg/kg of body weight. The drug substance included in this suspension contained more than 80% of particles  $< 10 \mu m$  in size. The remaining 6 animals acted as controls. Plasma oxendazole concentrations were

<sup>a</sup> NTZ-R, CSIRO Australia, NSW, Australia.

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

| Sheep No. | Period of study <sup>a</sup> |    |     |    |
|-----------|------------------------------|----|-----|----|
|           | 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  |

<sup>a</sup> 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 oxendazole); B = suspension B (milled oxendazole); C = suspension C (coarse oxendazole); D = suspension D (experimental formulation unrelated to this study).

monitored in the drenched sheep for 34 hours after drug administration. Oxendazole 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 oxendazole 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 ( $>95\%$ ) 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  $d_w$  value 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

PLASMA CONCENTRATION ( $\mu g/0.1 ml$ )

Fig 4—M of body w

TABLE 3 Dose of C

Plasma conc  
1 hr  
2 hr  
4 hr  
8 hr  
12 hr  
24 hr  
48 hr  
72 hr  
96 hr  
  
L<sub>50</sub> (hr)  
T<sub>max</sub> (hr)  
C<sub>max</sub> ( $\mu g/0.1$ )  
Total area  
  
m l  
L<sub>50</sub> =  
tration.

Fig 5—A

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

| Pairwise comparisons     |        |        |        |
|--------------------------|--------|--------|--------|
| Measurements             | A vs B | B vs C | A vs C |
| Plasma concentrations 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     |
| t <sub>1/2</sub>         | ns     | ns     | ns     |
| T <sub>max</sub>         | ns     | S      | ns     |
| C <sub>max</sub>         | ns     | ns     | ns     |
| Total area               | ns     | ns     | S      |

S = significant at P < 0.05. ns = not significant.

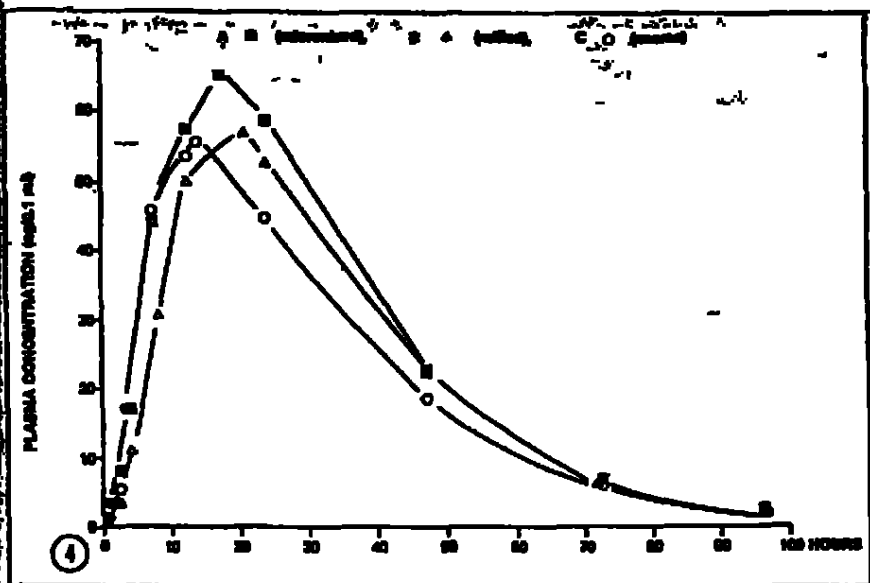


Fig 4—Mean oxidazepam plasma concentrations in sheep after an oral suspension dose (5 mg/kg of body weight) of 3 oxidazepam suspensions.

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

| Measurements                     | Formulation means         |                       |                       |
|----------------------------------|---------------------------|-----------------------|-----------------------|
|                                  | Suspension A (micronized) | Suspension B (milled) | Suspension C (coarse) |
| Plasma concentrations (ng/ml) at |                           |                       |                       |
| 1 hr                             | 2.77 (1.12)               | 0.80 (0.47)           | 1.19 (0.50)           |
| 2 hr                             | 7.58 (1.91)               | 3.48 (1.33)           | 4.77 (0.98)           |
| 4 hr                             | 16.80 (8.39)              | 10.80 (4.88)          | 16.80 (4.88)          |
| 8 hr                             | 43.80 (11.84)             | 30.80 (8.88)          | 47.40 (13.30)         |
| 12 hr                            | 57.50 (8.63)              | 48.80 (10.88)         | 58.30 (8.88)          |
| 24 hr                            | 68.30 (18.30)             | 82.30 (18.30)         | 45.10 (13.30)         |
| 48 hr                            | 23.80 (12.80)             | 22.80 (15.80)         | 15.80 (11.30)         |
| 72 hr                            | 6.32 (5.88)               | 4.08 (4.78)           | 4.68 (5.78)           |
| 96 hr                            | 1.88 (2.14)               | 1.88 (1.88)           | 1.88 (3.18)           |
| t <sub>1/2</sub> (hr)            | 12.30 (2.80)              | 12.30 (3.08)          | 12.80 (3.30)          |
| T <sub>max</sub> (hr)            | 18.80 (8.57)              | 31.00 (10.30)         | 14.00 (8.27)          |
| C <sub>max</sub> (ng/ml)         | 84.30 (14.37)             | 86.80 (12.18)         | 58.70 (18.68)         |
| Total area (ng/ml/hr)            | 2,822.00 (808)            | 2,388.00 (788)        | 2,121.00 (702)        |

ns in parentheses.

t<sub>1/2</sub> = biological half-life; T<sub>max</sub> = time to maximum plasma concentration; C<sub>max</sub> = maximum plasma concentration.



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

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, 8.20  $\mu$ m for lot B and 12.0  $\mu$ m for lot C. However, surface area values did not show similar differences, eg, in lot C for a dw of 12  $\mu$ m, the observed surface area value of 2.184 m<sup>2</sup>/g was excessively high, a value less than 1 m<sup>2</sup>/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  $\mu$ m) in addition to numerous relatively larger primary particles (10 to 12  $\mu$ m). In the SEM analyses, 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  $\mu$ m. 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 oxidazepam 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).  $\times 5,000$ .

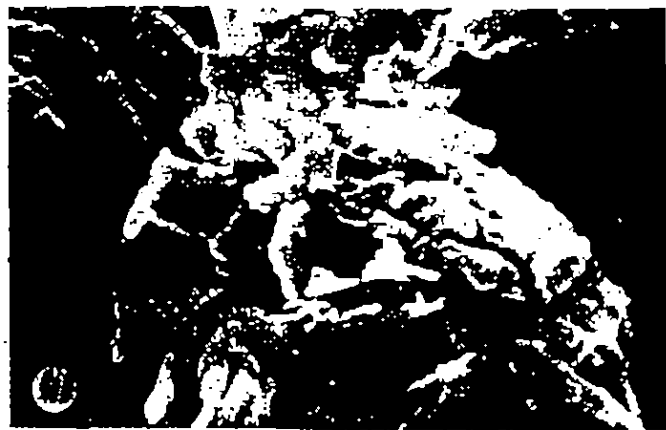


Fig 7—A SEM micrograph of coarse sample (lot C).  $\times 5,000$ .

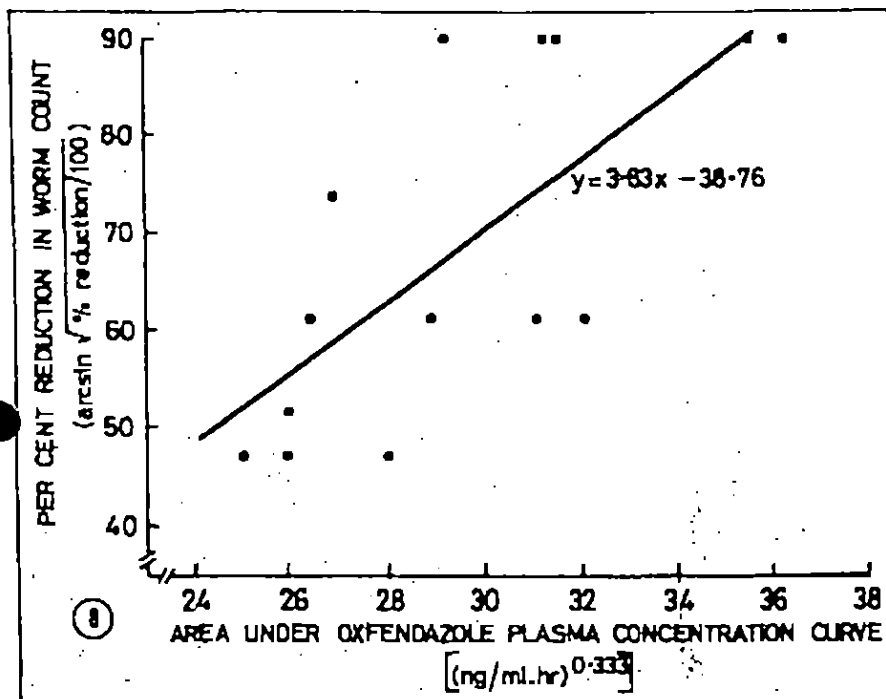


Fig 8—Linear regression of the reduction in count of *H. contortus*.

A was significantly different from suspension B and suspension C, and suspension B was significantly different from suspension C at  $T_1$  and  $T_2$ . After descending rank order, A, B, and C in that sequence, was observed. A preponderance of particles under  $8 \mu m$  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 disaggregate 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 567 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 disaggregated into

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 12.3 to 12.9 hours (mean 12.63  $\pm$  0.26 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 new material lots A, B, and C. This appears to explain the enhancement of dissolution rates observed with suspension A as compared with rates for suspensions B and C. In vitro dissolution data and in vivo sheep

plasma values 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.<sup>4</sup>

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.

<sup>4</sup> Anderson N, Lohy RH, Pritchard RK, 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 <sup>1</sup> | 0                  | 3            | 5                  | Difference <sup>2</sup> |
| K I <sup>3</sup>  | —                | 55.6 ± 8.3        | 58.4 ± 4.9 | 56.3 ± 8.9         |                         | 68.9 ± 19.6        | 107.7 ± 22.7 | 99.5 ± 34.9        |                         |
| K II <sup>3</sup> | —                | —                 | 70.8       | 118.6              |                         | —                  | 181.9        | 204.0              |                         |
| ML 1073           | 300              | —                 | 112.0      | 103.4              | (—)                     | —                  | 165.8        | 208.0              | (+/+)                   |
| ML 1077           | 300              | —                 | 117.0      | 119.2              | (—)                     | —                  | 190.4        | 230.2              | (+/+)                   |
| LM 16             | 125              | —                 | 109.8      | 108.2              | (—)                     | —                  | 189.8        | 142.8 <sup>4</sup> | (—)                     |
| K I <sup>3</sup>  | —                | 60.5 ± 10.0       | 124.7      | 181.4              |                         | 46.2 ± 8.9         | 88.9         | 94.5               |                         |
| Clofibrate        | 250              | —                 | 119.2      | 138.2 <sup>4</sup> | (+/+)                   | —                  | 52.7         | 106.4              | (+/+)                   |

<sup>1</sup> Control group (normal values after 0, 3 and 5 weeks; 0-values from 40 animals, all other values from 10 animals).

<sup>2</sup> Control group with hyperlipemic diet.

<sup>3</sup> Sign in brackets mean increase/decrease versus 3-week value (first line) or versus final value K II (second line).

<sup>4</sup> 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 Seiyaku, Co., Ltd., Osaka (Japan)

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

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

**Summary:** Pharmacokinetic behaviour of a new coronary vasodilator, the d-cis-isomer of 3-acetoxy-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(p-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride (diltiazem, CRD-401), as well as the bioavailability study of the release controlled tablet Herbezem<sup>®</sup> 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 clinical experiment, where diltiazem was administered i.v., 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 micronized 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-2,3-dihydro-5-[2-(dimethylamino)-Äthyl]-2-(p-methoxyphenyl)-1,5-benzothiazepin-4(5H)-on-Hydrochlorid (Diltiazem, CRD-401), und die Untersuchung seiner Bioverfügbarkeit aus einer Tablette mit verzögerter Freigabe (Herbezem<sup>®</sup>) 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.

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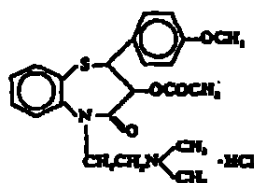
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il - Diltiazem

Bei den klinischen 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

## 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 deacetylN,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  $\mu$  and from 10 to 100  $\mu$ , respectively. These two crystalline forms and the powder obtained from crystal I with the particle size ranging from 5 to 30  $\mu$  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 J.P.X), pH 5.5 (0.05 M acetate buffer solution) and pH 7.5 (the second fluid for the disintegration test in J.P.X). 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: Tsumo Sanyaku Co. Ltd., Osaka (Japan).

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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.

### 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 polyethylene stoppered test tube. After centrifugation, the combined ether layer was evaporated to dryness in a tapered tube. The residue was dissolved in chloroform (70  $\mu$ l  $\times$  1, 50  $\mu$ l  $\times$  2) and was applied to TLC plate (Merck, Silica gel 60 F<sub>254</sub>). Spots for unchanged diltiazem and the other soluble metabolites, which had been developed in the solvent system of chloroform/n-butanol/ethanol/acetic acid (8:3:3:1:1), were detected and analyzed at 237 nm by using a densitometer (Hitachi Two-Wavelength Double Beam Spectrophotometer 354, 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  $\mu$ g of diltiazem in 1 ml of plasma can be exactly determined.

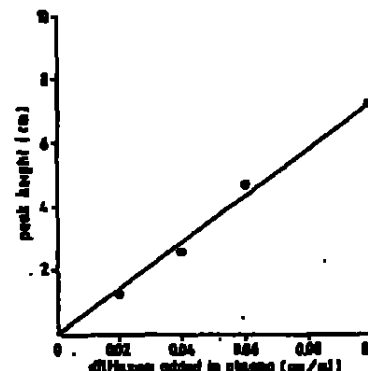


Fig. 1. Calibration curve for diltiazem added to plasma.

### 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  $t_n$  was calculated by using trapezoidal rule.  $t_n$  is the time which corresponds to seven times the cal-

where  $A_0$  and  $A_1$  are the fractions of initial 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.

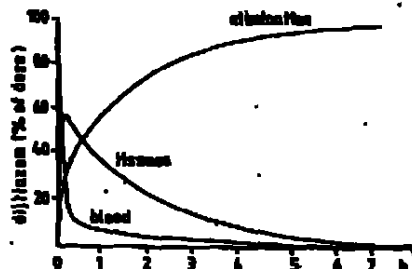


Fig. 4. Computed time course of distribution of diltiazem after i.v. administration of diltiazem hydrochloride according to two-compartment open model.

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).

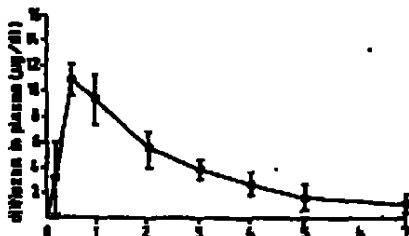


Fig. 5. Time course of plasma concentration of diltiazem following oral administration of 30 mg of diltiazem hydrochloride in the form of aqueous solution.

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.

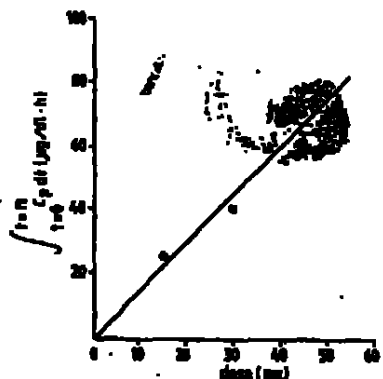


Fig. 6. Relationship between dose and AUC following oral administration of diltiazem hydrochloride in the form of aqueous solution.

Arizono, Furuta, / Drug Res. 27 (10), No. 7 (1977)  
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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.

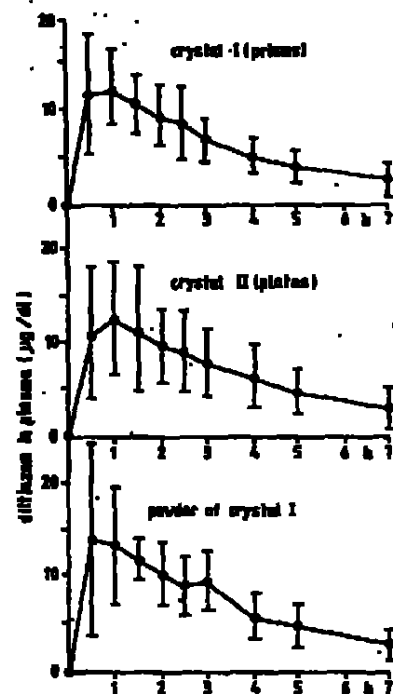


Fig. 7. Plasma concentrations of diltiazem following oral administration of 60 mg of diltiazem hydrochloride in three different forms of crystals.

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 | $C_{pmax}$ ( $\mu\text{g/dl}$ ) | $t_{max}$ (h) | AUC ( $\mu\text{g}\cdot\text{dl}^{-1}\cdot\text{h}$ ) |
|---------------------|------|---------------------------------|---------------|-------------------------------------------------------|
|                     |      | AV                              | AV            | AV                                                    |
| Crystal I           | C    | 9.5                             | 0.5           | 25.9                                                  |
|                     | D    | 17.6                            | 1.0           | 40.7                                                  |
|                     | E    | 16.7                            | 16.2          | 36.1                                                  |
|                     | F    | 17.0                            | 2.5           | 48.1                                                  |
|                     | G    | 20.3                            | 2.7           | 49.8                                                  |
|                     | H    | 16.2                            | 1.0           | 45.4                                                  |
| Crystal II          | C    | 16.4                            | 0.5           | 33.2                                                  |
|                     | D    | 18.8                            | 0.5           | 32.3                                                  |
|                     | E    | 16.0                            | 16.5          | 34.6                                                  |
|                     | F    | 17.1                            | 1.0           | 40.4                                                  |
|                     | G    | 19.6                            | 2.0           | 42.6                                                  |
|                     | H    | 19.2                            | 1.5           | 45.6                                                  |
| Powder of crystal I | C    | 15.9                            | 0.5           | 37.0                                                  |
|                     | D    | 16.9                            | 1.0           | 36.8                                                  |
|                     | E    | 18.3                            | 17.6          | 34.2                                                  |
|                     | F    | 21.9                            | 2.5           | 57.6                                                  |
|                     | G    | 18.7                            | 7.9           | 37.8                                                  |
|                     | H    | 19.8                            | 0.5           | 44.8                                                  |

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

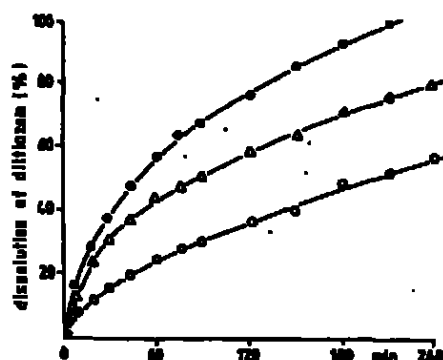


Fig. 8. Dissolution rate curves of diltiazem from the release-controlled tablet at different pH:  $\circ$ : pH 1.2;  $\Delta$ : pH 1.5;  $\square$ : pH 7.4.

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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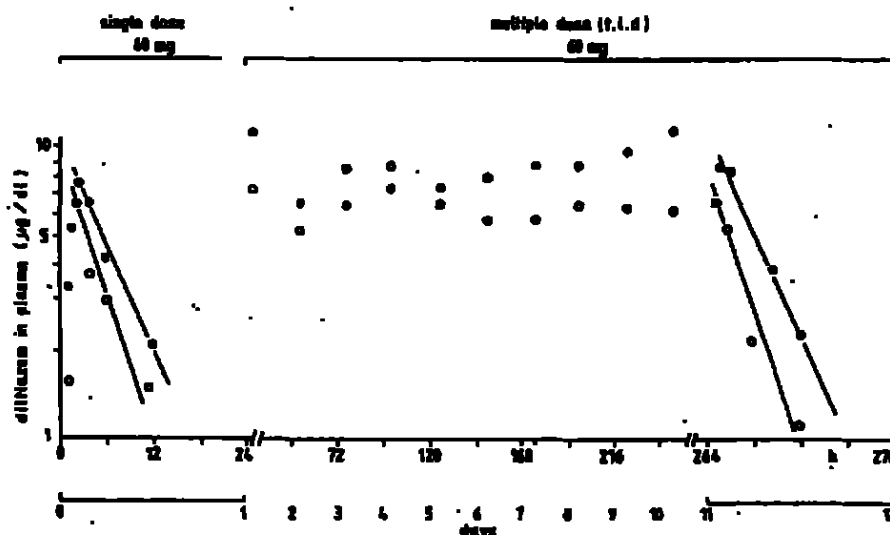


Fig. 9. Plasma levels of diltiazem after single and multiple administrations of the release-controlled tablet to two dogs.

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. Conasprem® 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 system 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 concentrate. Current brand leader formulation (Neoral®, Novartis, Switzerland) is in form of microemulsion concentrate 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; Vonderscher and Meisner, 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.

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

Based on the visual observation the novel system was denominated as gel based emulsion (GEM). 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 ( $-20^{\circ}\text{C}$ ).

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

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

$C_{\text{max}}$  and  $t_{\text{max}}$  were to be taken directly from the observed concentration–time data. The elimination rate constant ( $k_{\text{el}}$ ) 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 ( $\text{AUC}_{(0-t)}$ ) 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  $t_{\text{max}}$  and  $t_{1/2}$ , arithmetic means, standard deviations, and 95%-confidence intervals for means were to be calculated. For  $C_{\text{max}}$ ,  $\text{AUC}_{(0-t)}$ ,  $\text{AUC}_{(0-\infty)}$ , and  $\text{Frel}$ , geometric means, standard deviations, and 95%-confidence intervals for means were to be calculated.

The primary variables  $\text{AUC}_{(0-\infty)}$  and  $C_{\text{max}}$ , 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  $C_{\text{max}}$ , respectively.

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**Table 1**  
Summary of pharmacokinetic data

| Parameter                       | <i>t</i> <sub>1/2</sub> (h) | <i>t</i> <sub>max</sub> (h) | <i>C</i> <sub>max</sub> (µg/ml) | AUC <sub>(0–t)</sub> (ng h/ml) | AUC <sub>(0–∞)</sub> (ng h/ml) |
|---------------------------------|-----------------------------|-----------------------------|---------------------------------|--------------------------------|--------------------------------|
| <b>Formulation A</b>            |                             |                             |                                 |                                |                                |
| Arithmetic mean                 | 6.24                        | 1.33                        | 1372.69                         | 4631.71                        | 4661.85                        |
| S.D.                            | 1.3                         | 0.33                        | 351.28                          | 1204.56                        | 1241.87                        |
| Geometric mean                  | 6.12                        | 1.3                         | 1229.84                         | 4483.35                        | 4712.35                        |
| Minimum                         | 4.06                        | 1                           | 908.1                           | 2635.32                        | 2873.57                        |
| Maximum                         | 8.24                        | 2                           | 1930.3                          | 6432.76                        | 6684.33                        |
| <b>Formulation B</b>            |                             |                             |                                 |                                |                                |
| Arithmetic mean                 | 6.41                        | 1.5                         | 1196.49                         | 4438.33                        | 4696.56                        |
| S.D.                            | 1.3                         | 0.48                        | 308.26                          | 1082.91                        | 1143.13                        |
| Geometric mean                  | 6.28                        | 1.43                        | 1161.84                         | 4326.15                        | 4576.94                        |
| Minimum                         | 4.21                        | 1                           | 851.8                           | 3158.66                        | 3254.08                        |
| Maximum                         | 8.93                        | 2.5                         | 1725                            | 6206.13                        | 6643.15                        |
| <b>Formulation, Cholestanol</b> |                             |                             |                                 |                                |                                |
| Arithmetic mean                 | 6.13                        | 1.33                        | 1358.95                         | 4647.01                        | 4687.33                        |
| S.D.                            | 1.32                        | 0.33                        | 380.35                          | 1204.41                        | 1430.5                         |
| Geometric mean                  | 5.99                        | 1.3                         | 1307.19                         | 4472.8                         | 4703.35                        |
| Minimum                         | 3.92                        | 1                           | 820.7                           | 2933.47                        | 3028.39                        |
| Maximum                         | 7.87                        | 2                           | 1803.3                          | 7330.08                        | 7686.89                        |

**Table 2**  
Bioequivalence criteria

|                         |                  | Lower 90%-confidence limit | Point estimate | Upper 90%-confidence limit |
|-------------------------|------------------|----------------------------|----------------|----------------------------|
| AUC <sub>(0–∞)</sub>    | Form A/reference | 0.93                       | 1.00           | 1.08                       |
|                         | Form B/reference | 0.88                       | 0.97           | 1.07                       |
| AUC <sub>(0–t)</sub>    | Form A/reference | 0.93                       | 1.00           | 1.09                       |
|                         | Form B/reference | 0.87                       | 0.97           | 1.07                       |
| <i>C</i> <sub>max</sub> | Form A/reference | 0.90                       | 1.02           | 1.16                       |
|                         | Form B/reference | 0.79                       | 0.89           | 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<sub>(0–∞)</sub> and AUC<sub>(0–t)</sub> (*P* < 0.05). For the AUC- and *C*<sub>max</sub>-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.

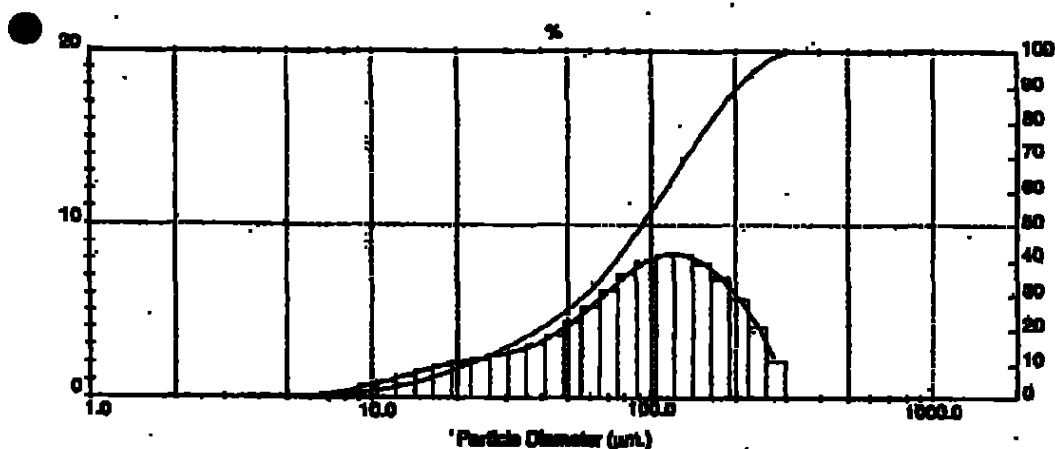
These findings interfere with previous concept that smaller is better.

Nevertheless, improvements in bioavailability which consequent on better dispersion abilities besides to Neoral® (e.g. giving smaller particles after administration) was described with Cosuper® (emulsion giving formulation; Galena, Czech Republic; Noble and Martham, 1995) and SangCys® (microdispersion giving formulation; SangStat, USA; Duchin, 1997; Casafax 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

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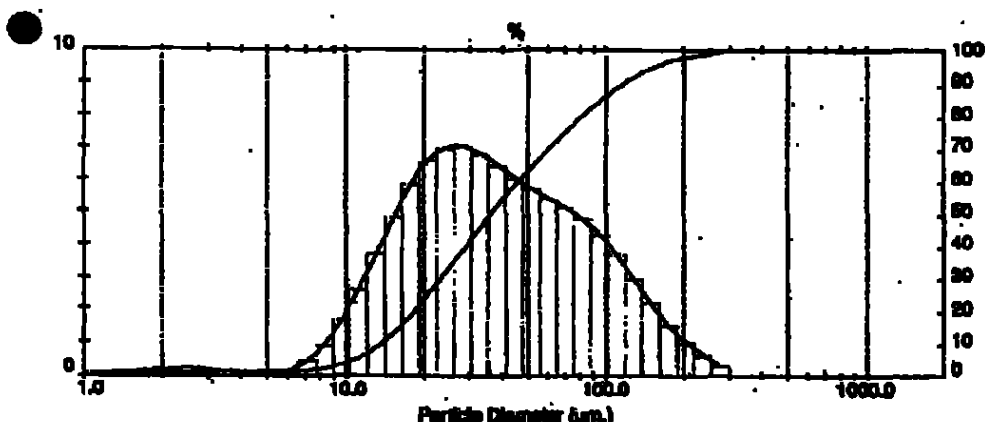
(a) formulation A- Gem 101



Melvern Instruments Ltd.  
Malvern, UK  
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MasterSizer Micro Ver. 2.18  
Serial Number: 34044-02

12 May 99

(b) formulation B- Gem 304



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Serial Number: 34044-02

12 May 99 12:20

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 Fiedorik, 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.

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

|                             | RE diameter (µm)  | CV%  |
|-----------------------------|-------------------|------|
| Sandimmune®                 | 3.73 <sup>a</sup> | 61.3 |
| Cosagra®                    | 1.73 <sup>a</sup> | 22.3 |
| Cyclosporine cps (BON Lab.) | 0.95 <sup>a</sup> | 24.7 |
| Cosagra® cps                | 0.94 <sup>a</sup> | 19.5 |
| SengCyA®                    | 0.85 <sup>b</sup> | 15.1 |
| Neoral®                     | 0.87 <sup>b</sup> | 12.9 |

RE, diameter, effective diameter; CV%, coefficient of variation (%).

<sup>a</sup> Optical microscope technique; computer analysis by Leica®, Laboratory Imaging Inc.

<sup>b</sup> Dynamic laser scattering performed on BI 200SM, BIV000 AT digital correlator (Brookhaven Inc.) with argon 514 nm laser (Laser Laser Inc.).

2. *Membrane*—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 (Svensson et al., 1994). Disruption of the cellular tight junction is another potential effect of excipients observed on Caco-2 cells model (Narverkar et al., 1996). More likely than effect on tight junction the affection 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 coherence with application of Cremophor RH 40, laethidol-tarcholet 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 (Ismailov 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  $394 \pm 35$  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<sup>a</sup>

| 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                    |

<sup>a</sup> 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.

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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

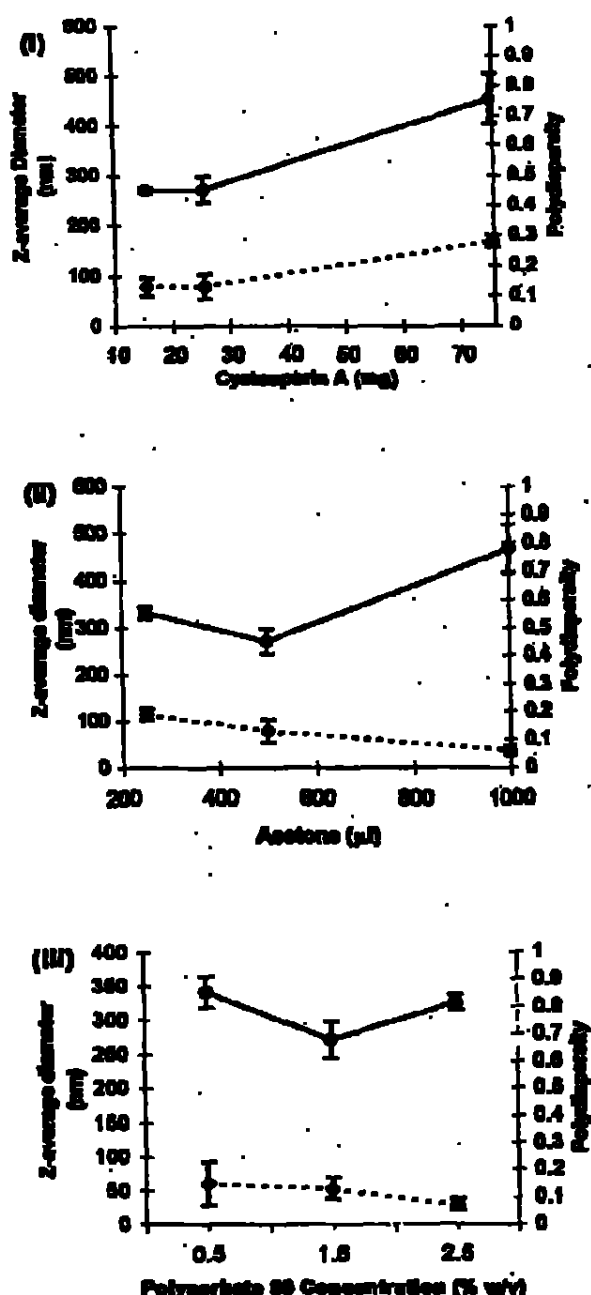


Fig. 1. Effect of varying (I) cyclosporin A (CYA) quantity, (II) acetone volume, and (III) polysorbate 80 concentration upon particle size (●) and polydispersity (◆) of CYA nanospheres. Each data point represents the mean of three batches with standard deviations shown.

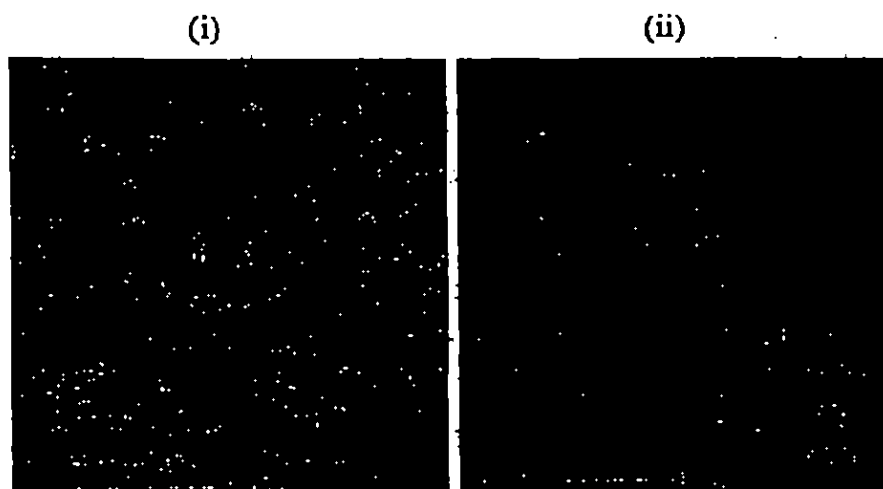
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Fig. 2. Scanning electron micrographs of cyclosporin A (CYA) nanospheres with mean Z-average diameters of (i) 352 nm and (ii) 824 nm.

Table 2

Pharmacokinetic parameters for cyclosporin A (CYA) nanospheres and Neoral<sup>®</sup> given to dogs as a single oral dose of 100 mg CYA<sup>a</sup>

| Formulation                       | AUC (ng h/ml)             | Initial $C_{max}$ (ng/ml) | $T_{max}$ (h)          |
|-----------------------------------|---------------------------|---------------------------|------------------------|
| CYA nanospheres                   | 351 (20%) <sup>b</sup>    | 25 (58%) <sup>b</sup>     | 2.2 (36%) <sup>b</sup> |
| Neoral <sup>®</sup> microemulsion | 12 790 (16%) <sup>c</sup> | 1689 (26%) <sup>c</sup>   | 1.9 (37%) <sup>c</sup> |

<sup>a</sup> Relative standard deviations are shown in brackets.

<sup>b</sup>  $n = 5$ .

<sup>c</sup>  $n = 10$ .

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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