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Señor Director

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Superintendencia de Industria y Comercio

E. S. D.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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Trámite: 011 Evento: 378 Actuación: 449

Referencia	:	Expediente **No. 13.236.457**
Asunto	:	Solicitud de patente para: "**FORMULACIÓN QUE COMPRENDE DERIVADO DE FENILAMINOPIRIMIDINA COMO AGENTE ACTIVO**".
Solicitante	:	NATCO PHARMA LIMITED
Fecha de solicitud	:	4 de octubre de 2013

1. ALEGATOS CONTESTACIÓN OPOSICIÓN

Yo, Luz Clemencia de PAÉZ, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, por el presente escrito doy respuesta a la oposición formulada por la sociedad **LAFRANCOL S.A.**, opositora en el asunto de la referencia.

2. CONTESTACIÓN DENTRO DEL TÉRMINO LEGAL

El oficio **No. 1288**, 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 3 de noviembre de 2015. El 27 de enero de 2015 se pidió plazo adicional, por lo que la presente defensa es presentada en tiempo oportuno.

3. 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 declaren sin fundamento la oposición presentada por la sociedad **LAFRANCOL S.A.**

En consecuencia, se declare que el invento titulado "**FORMULACIÓN QUE COMPRENDE DERIVADO DE FENILAMINOPIRIMIDINA COMO AGENTE ACTIVO**" cumple con los requisitos exigidos en el artículo 14, 16 y 18 de la Decisión 486 de la Comisión de la Comunidad Andina y demás normas concordantes.

4. FUNDAMENTO FÁCTICO DE LA OPOSICIÓN

A continuación procederé a enumerar los argumentos que la opositora **LAFRANCOL S.A.** considera pertinentes para presentar oposición a la presente solicitud son los siguientes:

4.1 Las composiciones y formulaciones farmacéuticas que una cantidad efectiva de NRC-AN-019, sus sales y polimorfos de forma (I), (II) y (III) son totalmente conocidas en la industria farmacéutica y lo anterior, se puede evidenciar en diversas publicaciones internacionales (por ejemplo WO2006/027795, US2009/227611, WO2009/109867, US2008/306100 y *Antitumor activity of NRC-AN-019 in a preclinical breast cáncer model*). No existe ninguna novedad sobre este precepto y mucho menos nivel inventivo.

4.2 Seguidamente, la parte opositora señala que las composiciones reivindicadas involucran compuestos como el Imatinib, ya conocidos en el estado del arte y trae a colación referencias realizadas sobre dichos compuestos en "The Merck index" y en distintas solicitudes de patentes.

4.3 Finalmente, la parte opositora asegura que la presente solicitud pretende patentar una forma cristalina, la cual no se considera patentable a la luz de la normativa andina.

5. MODIFICACIONES AL PLIEGO REIVINDICATORIO

El solicitante decide aportar un nuevo capítulo reivindicatorio conformado por 26 reivindicaciones en las que se han incluido las siguientes modificaciones:

5.1 Se han especificado las cantidades de cada componente en la reivindicación 1, de acuerdo con la información divulgada en la antigua reivindicación 2.

5.2 La fase lipofílica se ha definido de manera que comprende un triglicérido, un diglicérido, un monoglicérido, un ácido graso C12 a C22, o una mezcla de los mismos, dicho triglicérido, diglicérido o monoglicérido contiene residuos de ácido graso C12 a C22 unidos a al

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menos un grupo alcohol en la porción glicérica, con base en las antigua reivindicación 4 y en las enseñanzas de la descripción de la presente solicitud.

5.3 El al menos un surfactante principal farmacéuticamente aceptable se ha definido por comprender un aceite vegetal polioxietilen glicolado, con base en la antigua reivindicación 9.

5.4 El solvente miscible con agua farmacéuticamente aceptable se ha definido como un derivado de α-hidroxi-ω -hidroxipoli(oxi-1,2-etanodiilo), etanol o alcohol bencílico, con base en la antigua reivindicación 13.

5.5 Se ha incluido el nombre completo de NRC-AN-019 en la antigua reivindicación 3.

5.6 Se ha limitado el co-tensoactivo en la antigua reivindicación 8 a polioxilglicéridos de caprilocaproilo, con base en la antigua reivindicación 10.

5.7 La antigua reivindicación 17 (nueva reivindicación 26) se ha modificado de manera similar a la reivindicación 1.

5.8 Las antiguas reivindicaciones 2, 4 y 11 a 13 han sido eliminadas.

5.9 Las anteriores modificaciones se encuentran debidamente soportadas en la descripción como se muestra a continuación:

5.9.1 Un número de fases lipofílicas han sido ejemplificadas en la solicitud: ácido oleico (por ejemplo, los Ejemplos 1 a 34); ácido ricinoleico (por ejemplo, Ejemplos 35 a 37); triglicéridos de cadena media (por ejemplo, Ejemplos 36, 38, 39); aceite de linaza (Ejemplo 37); glicerol monocaprilocaprato (Ejemplos 44 a 46); los aceites de oliva, girasol y canola (Ejemplos 49); y monooleato de glicerilo (Ejemplos 50 a 52).

5.9.2 Un número de diferentes agentes tensioactivos se han ejemplificado en la solicitud: polioxil 35 aceite de ricino (todos los Ejemplos), glicéridos de caprilocaproilo polioxil-8 (por ejemplo, Ejemplos 23 a 25), laureth sulfato de sodio (por ejemplo, Ejemplo 39) y glicerol monocaprilocaprato (por ejemplo, Ejemplos 35, 36 y 38).

5.9.3 Un número de diferentes co-tensioactivos se han ejemplificado en la solicitud: glicéridos de caprilocaproilo polioxil-8 (por ejemplo, Ejemplo 4), glicéridos caprílicos (por

ejemplo, Ejemplo 5), monooleato de sorbitán (por ejemplo, Ejemplo 6) y el linoleato de PEG-6-glicerilo (por ejemplo, Ejemplo 7).

5.10 Las nuevas reivindicaciones constituyen claramente una generalización razonable de los ejemplos de la solicitud. Como se mencionará a continuación, se ha encontrado sorprendentemente que el NRC-AN-019, un compuesto que no es lipofílico y que no tiene solubilidad en agua, puede ser formulado exitosamente en una composición que comprende una fase lipofílica, un tensoactivo y un solvente. Esta composición inventiva está soportada a través de todo su alcance en los ejemplos de la solicitud, demosntrando que un rango de componentes puede ser empleado para formular el NCR-AN-019.

Estas modificaciones se ajustan al artículo 34 de la Decisión 486 de 2000 y no constituyen una ampliación de la protección que correspondería a la divulgación contenida en la solicitud inicial.

A pesar de eliminar materia del pliego reivindicatorio originalmente presentado, mi representada no renuncia a la protección que pueda obtener sobre dicha materia y se reserva el derecho de presentar una solicitud divisional que la contenga durante el trámite de la presente solicitud.

6. RESPUESTA A LOS HECHOS Y ALEGATOS

A continuación, me permito presentar a consideración de este Despacho, las razones que demuestran que es procedente otorgar privilegio de patente a la invención solicitada por mi representada en este asunto, toda vez que no es anticipada por los documentos del estado del arte citados por los apoderados de las partes opositoras como sustento de sus pretensiones.

6.1 Con respecto al fundamento de la oposición

6.1.1 La parte opositora únicamente se refiere al arte previo citado en el Reporte de Búsqueda Internacional. No presenta una discusión específica de ninguna de las divulgaciones de los documentos citados, sino que meramente presenta argumentos vagos mencionando que los mismos se encuentran relacionados con la materia de la presente invención.

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6.1.2 La parte opositora ha mencionado que la composición que se reivindica comprende imatinib, el cual es un compuesto conocido y patentado (US 5,521,184 y EP 0564409), siendo estos documento relevantes para la novedad de la presente solicitud. Estas afirmaciones son incorrectas, teniendo en cuenta que la composición de la presente solicitud no comprende imatinib, sino que comprende NRC-AN-019. También vale la pena resaltar que aun cuando la composición comprendiera imatinib, el hecho de que dicho compuesto esté patentando es irrelevante para la novedad de la composición reclamada.

6.1.3 No existen comentarios específicos en la oposición acerca de si es conocido u obvio formular un medicamento tal como el NRC-AN-019 utilizando una formulación de sistema de entrega de drogas auto-emulsificante (SEDDS). El opositor sólo realiza un planteamiento acerca de la "práctica común."

6.1.4 La parte opositora menciona que los polimorfos no son materia patentable. Sin embargo se desea aclarar que la *GUÍA PARA EXAMEN DE SOLICITUDES DE PATENTE DE INVENCIÓN Y MODELO DE UTILIDAD* define el polimorfismo y establece todos los lineamientos para que el Examinador pueda realizar un correcto estudio de patentabilidad sobre los mismos, toda vez que los polimorfos sí son materia patentable.

6.2 Con respecto a la claridad de las reivindicaciones

6.2.1 Los términos "preferiblemente" y "más preferiblemente" se han eliminado de las reivindicaciones, ubicando las características preferidas en reivindicaciones dependientes.

6.3 Con respecto a la novedad y al nivel inventivo

6.3.1 Contrario a lo que afirma la parte opositora, la composición tal como se encuentra reivindicada en la presente solicitud no se encuentra divulgada en ninguna anterioridad. Consecuentemente, la invención si cumple con el requisito de novedad.

6.3.2 La persona medianamente versada en la materia no hubiera encontrado motivación alguna en el arte previo para pensar que utilizar una fase lipofílica, un tensoactivo y un solvente fuera aplicable a un compuesto no lipofílico, que no es soluble en agua, como el NRC-AN-019.

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6.3.3 La presente invención pretende incrementar la biodisponibilidad del NRC-AN-019. Este compuesto es completamente diferente de los compuestos divulgados en los documentos señalados por la parte opositora. La molécula NRC-AN-019 no es lipofílica y es completamente hidrofóbica (con ninguna solubilidad en agua).

6.3.4 Usualmente, un compuesto dado, con solubilidad limitada en agua tendería a tener una naturaleza lipofílica y a tener buena solubilidad en solventes orgánicos no-polares (y viceversa). Sin embargo, esto no es una regla universal. Por consiguiente, existen algunos compuestos que tiene buena solubilidad tanto en solventes acuosos como en solventes orgánicos no-polares. Similarmente, hay compuestos que tienen una muy baja (o ninguna) solubilidad en ambos tipos de solventes. El compuesto NRC-AN-019 cae dentro de esta última categoría. La evidencia para esto se puede ver en los resultados experimentales que se anexan a este memorial, en la declaración de Durga Maheswari Parvataneni.

6.3.5 La declaración confirma que el NRC-AN-019 tiene una naturaleza completamente hidrofóbica, no siendo soluble en agua. Por consiguiente, aun después de 4 horas de agitación en agua, no se detectó NRC-AN-019 en absoluto. (Página 3, Resultado)

6.3.6 La declaración confirma que el NRC-AN-019 también tiene baja solubilidad en los solventes orgánicos no-polares 1-octanol, n-hexano, tolueno y ciclohexano. Por consiguiente, la solubilidad varió entre 0.22 a 535.4848 µg/mL (Página 4 de la declaración). Con base en una densidad de 0.824 g/mL para el 1-octanol y 0.779 g/mL para el ciclohexano, esto significa que el número de partes por peso del solvente orgánico necesario para disolver una parte de NRC-AN-019 estuvo entre 1500 a aproximadamente 3,500,000.

6.3.7 La declaración confirma que el NRC-AN-019 también tiene una baja solubilidad en lípidos. Por consiguiente, su solubilidad en varios lípidos varió entre 0.06 a 3.27 mg/mL (ver páginas 7 a 11). Esto significa que el número de partes por peso de lípido necesario para disolver una parte de NRC-AN-019 varía entre aproximadamente 300 a 15,000.

6.3.8 Siguiendo la nomenclatura discutida en los extractos de la US Pharmacopoeia que se anexa igualmente a este memorial, la solubilidad del NRC-AN-019 en solventes orgánicos no-polares entonces varía entre "muy poco soluble" a "prácticamente insoluble" (o simplemente "insoluble"), y su solubilidad en lípidos varía entre "ligeramente soluble" a "prácticamente insoluble" (u otra vez, simplemente "insoluble").

6.3.9 Como podrá apreciar el Examinador, los datos señalados aquí arriba muestran que el compuesto NRC-AN-019, es fundamentalmente diferente a los compuestos de los documentos que cita la parte opositora: NRC-AN-019 no es lipofílico y es sustancialmente menos soluble en medio orgánico. Adicionalmente, el NRC-AN-019 no tiene solubilidad en agua, de manera que es completamente hidrofóbico.

6.3.10 Teniendo en cuenta las diferencias fundamentales entre el NRC-AN-019 y los compuestos lipofílicos formulados en los documentos citados, no es creíble sugerir que cualquier persona versada en la materia hubiera contemplado tomar dicho paso con alguna expectativa razonable de éxito.

6.3.11 Por las razones expuestas anteriormente, se concluye que la materia reivindicada no es obvia y por consiguiente cumple con el requisito de nivel inventivo.

6.4 Concesión de solicitudes equivalentes

Se desea poner de presente que la solicitud equivalente EP2683381, cuyas reivindicaciones tienen un alcance mayor que las reivindicaciones de la presente solicitud, ha sido concedida por la Oficina Europea de Patentes (EPO).

7. PETICIONES

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

7.1 Declarar infundada la oposición presentada por **LAFRANCOL S.A.**

7.2 Declarar que el invento titulado "**FORMULACIÓN QUE COMPRENDE DERIVADO DE FENILAMINOPIRIMIDINA COMO AGENTE ACTIVO**" para el cual mi representada solicita el otorgamiento de la patente de invención, cumple con los requisitos de los artículos 14, 16 y 18 concordantes de la Decisión 486 de 2000 y otorgar el privilegio de patente solicitado.

8. NOTIFICACIONES

Recibiré notificaciones en la Secretaría de su Despacho o en mi oficina de abogada situada en la Carrera 4 72-35, piso 4 de Bogotá.

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9. ANEXOS

- Nuevo pliego reivindicatorio conformado por 26 reivindicaciones;
- Formulario para modificaciones;
- Recibo por concepto de modificaciones;
- Declaración técnica de Durga Maheswari Parvataneni;
- *US Pharmacopeia*;
- Porter, et al., *Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs*

Señor Director,

LUZ CLEMENCIA DE PÁEZ

C.C. 35.456.344 de Usaquén

T.P.A. No. 23.555

COLO-17766-0841-0003-4
COLO-17766-0264-0002-4
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No. M-2016-287768\GCH

Industria y Comercio
SUPERINTENDENCIA

Espacio reservado para el adhesivo de radicación

DIRECCIÓN DE NUEVAS CREACIONES MODIFICACIONES Y/O CORRECCIONES

1. **Identificación del Tramite**

- ☐ **Error material**
- ☒ **Modificación a una Solicitud en trámite**
- ☒ Patente de Invención
- ☐ Patente de Modelo de Utilidad
- ☐ Registro de Diseño Industrial
- ☐ Esquema de Trazado de Circuitos Integrados

2. **Datos del solicitante**

Persona Natural ☐ Persona Jurídica ☒

NATCO PHARMA LIMITED

Nombre o denominación / Nombre o razón social completa

Nombre representante legal

Tipo de empresa Micro ☐ Pequeña ☐ Mediana ☐ Otra ☐

Documento de identificación: ☐ C.C. ☐ C.E. ☐ NIT ☐ Otro Número_____

Nacionalidad/País de constitución	Dirección y domicilio del titular	Ciudad
India	Natco House, Road No. 2, Banjara Hills, Hyderabad 500 033, Andhra Pradesh, India	Andhra Pradesh
Dirección electrónica	**No. Fax**	**Número telefónico**
clientes@cavelier.com	2 11 86 50	3 47 36 11

☐ **Autorizo expresamente a la Superintendencia de Industria y Comercio para que todas las comunicaciones sean enviadas a través de la dirección de correo electrónico**

3. **Datos del apoderado:**

Apellidos y Nombre	Documento de identificación	Tarjeta profesional
De Páez Luz Clemencia	C.C. 35.456.344	T.P.A. 23.555

Dirección y domicilio		Ciudad
Carrera 4ª No. 72 –35		Bogotá
Dirección electrónica	**No. Fax**	**Número telefónico**
cavelier@cavelier.com	2 11 86 50	3 47 36 11
En caso de haber presentado poder general para asuntos que se adelanten ante la Delegatura de Propiedad Industrial, sírvase indicar el número de su radicación o protocolo de poder general		

4. Datos del trámite

Corrección:

Expediente No 13.236.457

Aparece

Debe ser

Modificación:

☒ **Modificación al capítulo reivindicatorio.**
Presento nuevo capítulo reivindicatorio compuesto por 26 reivindicaciones y pido respetuosamente que el examen de patentabilidad sea realizado sobre estas nuevas reivindicaciones presentadas. Respetuosamente manifiesto que a pesar de eliminar materia del pliego reivindicatorio originalmente presentado, mi representada no renuncia a la protección que pueda obtener sobre dicha materia y además se reserva el derecho de presentar una solicitud divisional que la contenga durante el trámite de la presente solicitud, beneficiándose de la fecha de prioridad inicialmente reivindicada.

☐ **Modificación al capítulo descriptivo.**

☐ **Modificación al resumen.**

☐ **Modificación a los dibujos.**

5. Anexos

☒ Comprobante de pago de la tasa para la presentación de la corrección y/o modificación por $164.000.

☐ Poder, si fuere el caso.

☒ Documento que contiene las modificaciones o correcciones.

☐ Copia de la solicitud y sus anexos en formato magnético.

6. Firma	
Nombre y apellidos **LUZ CLEMENCIA DE PAEZ**	Firma Luz Clemencia de Paez
C.C. 35.456.344 DE Usaquén	Tarjeta Profesional 23.555

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REIVINDICACIONES

1. Una composición farmacéutica para administración oral que comprende:

(a) una cantidad terapéuticamente efectiva de (3,5-bis-trifluorometil)-N-[4-metil-3-(4-piridin-3-il-pirimidin-2-ilamino)fenil]benzamida (NRC-AN-019) o una sal farmacéuticamente aceptable del mismo en una cantidad de 0.25 a 10% en peso con respecto al peso total de la composición;

(b) una fase lipofílica en una cantidad de 12.5 a 65% en peso con respecto al peso total de la composición;

(c) por lo menos un agente tensoactivo principal farmacéuticamente aceptable en una cantidad de 8 a 60% en peso con relación al peso total de la composición; y

(d) un solvente miscible con agua farmacéuticamente aceptable en una cantidad de 5.5 a 25% por peso con relación al peso total de la composición,

en donde:

la fase lipofílica comprende un triglicérido, un diglicérido, un monoglicérido, un ácido graso C_{12} a C_{22}, o una mezcla de los mismos, dicho triglicérido, diglicérido o monoglicérido contiene residuos de ácido graso C_{12} a C_{22} unidos a al menos un grupo alcohol en la porción glicérica;

el al menos un surfactante principal farmacéuticamente aceptable comprende un aceite vegetal polioxietilen glicolado; y

el solvente miscible con agua farmacéuticamente aceptable es un derivado de α-hidroxi-ω -hidroxipoli(oxi-1,2-etanodiilo), etanol o alcohol bencílico.

2. La composición farmacéutica de conformidad con la reivindicación 1, que comprende los polimorfos Forma I, Forma II o Forma III de (3,5-bis-trifluorometil)-N-[4-metil-3-(4-piridin-3-il-pirimidin-2-ilamino)-fenil]-benzamida (NRC-AN-019).

3. La composición farmacéutica de conformidad con la reivindicación 1, en donde el ácido graso C_{12} a C_{22} es un ácido graso mono-insaturado de C_{12-20} que es líquido a temperatura ambiente.

4. La composición farmacéutica de conformidad con la reivindicación 1, en donde el ácido graso C_{12} a C_{22} es ácido (Z)-9-octadecenoico.

5. La composición farmacéutica de conformidad con cualquiera de las reivindicaciones anteriores, en donde los residuos de ácidos grasos insaturados de C_{18} están presentes en la

fase lipofílica en una cantidad de por lo menos 20% en peso en relación con el peso total de la fase lipofílica.

6. La composición farmacéutica de conformidad con la reivindicación 5, en donde los residuos de ácidos grasos insaturados de C_{18} están presentes en la fase lipofílica en una cantidad de por lo menos 40% en peso en relación con el peso total de la fase lipofílica.

7. La composición farmacéutica de conformidad con cualquiera de las reivindicaciones anteriores, en donde los residuos de ácido graso saturado están presentes en la fase lipofílica en una cantidad de 50% en peso o menos, con base en el peso total de la fase lipofílica.

8. La composición farmacéutica de conformidad con cualquiera de las reivindicaciones anteriores, en donde el por lo menos un agente tensoactivo comprende el agente tensoactivo principal y uno o más co-agentes tensoactivos, en donde el uno o más co-agentes tensoactivos comprenden polioxilglicéridos de caprilocaproilo.

9. La composición farmacéutica de conformidad con cualquiera de las reivindicaciones anteriores, en donde el aceite vegetal glicolado de polioxietileno es un aceite de ricino glicolado de polioxietileno.

10. La composición farmacéutica de conformidad con la reivindicación 8 ó 9, en donde el aceite de ricino glicolado de polioxietileno es aceite de ricino de polioxilo 35.

11. La composición farmacéutica de conformidad con la reivindicación 8, 9 o 10, en donde el co-agente tensoactivo está presente en una cantidad de 5 a 45% en peso en relación con el peso total de la composición.

12. La composición farmacéutica de conformidad con cualquiera de las reivindicaciones anteriores, en donde el derivado de α-hidroxi-ω-hidroxipoli(oxi-1,2-etanodiilo) es polietilenglicol 600.

13. La composición farmacéutica de conformidad con cualquiera de las reivindicaciones anteriores, que comprende además un antioxidante.

14. La composición farmacéutica de conformidad con la reivindicación 13, en donde el antioxidante se selecciona de hidroxianisol butilado, hidroxitolueno butilado y una mezcla de los mismos.

15. La composición farmacéutica de conformidad con la reivindicación 13, en donde el antioxidante es una mezcla de hidroxianisol butilado e hidroxitolueno butilado en una relación de 6:4 a 9:1.

16. La composición farmacéutica de conformidad con la reivindicación 13, en donde el antioxidante es una mezcla de hidroxianisol butilado e hidroxitolueno butilado en una relación de 7:3 a 8:2.

17. La composición farmacéutica de conformidad con cualquiera de las reivindicaciones anteriores, que comprende además un agente edulcorante.

18. La composición farmacéutica de conformidad con la reivindicación 17, en donde el agente edulcorante es sacarina.

19. La composición farmacéutica de conformidad con la reivindicación 17, en donde el agente edulcorante es sacarina en una cantidad de 0.1% en peso a 10% en peso en relación con el peso total de la composición.

20. La composición farmacéutica de conformidad con la reivindicación 17, en donde el agente edulcorante es sacarina en una cantidad de 0.25% en peso a aproximadamente 5% en peso en relación con el peso total de la composición.

21. La composición farmacéutica de conformidad con cualquiera de las reivindicaciones anteriores, que comprende además un agente saborizante.

22. La composición farmacéutica de conformidad con la reivindicación 21, en donde el agente saborizante se selecciona de sabores a base de aceite, ya sea individualmente o en combinación.

23. La composición farmacéutica de conformidad con la reivindicación 21 ó 22, en donde el agente saborizante está presente en una cantidad de 0.1% en peso a aproximadamente 10% en peso en relación con el peso total de la composición.

24. La composición farmacéutica de conformidad con la reivindicación 21 ó 22, en donde el agente saborizante está presente en una cantidad de 0.25% en peso a 1% en peso en relación con el peso total de la composición.

25. La composición farmacéutica de conformidad con cualquiera de las reivindicaciones anteriores, que está en forma de una solución, un gel blando, o una formulación de cápsula de coraza dura.

26. Un procedimiento para producir una composición farmacéutica como se define en cualquiera de las reivindicaciones 1 a 25, dicho procedimiento comprende:

- mezclar (3,5-bis-trifluorometil)-N-[4-metil-3-(4-piridin-3-il-pirimidin-2-ilamino)fenil] benzamida (NRC-AN-019) en una cantidad de 0.25 a 10% en peso con respecto al peso total de la composición con un solvente miscible con agua en una cantidad de 5.5 a 25% por peso con relación al peso total de la composición,
- añadir una fase lipofílica en una cantidad de 12.5 a 65% en peso con respecto al peso total de la composición,
- añadir un agente tensoactivo principal en una cantidad de 8 a 60% en peso con relación al peso total de la composición y opcionalmente un co-tensoactivo en una cantidad de 5 a 45% en peso en relación con el peso total de la composición, y
- agitar con la aplicación de calor,

en donde:

la fase lipofílica comprende un triglicérido, un diglicérido, un monoglicérido, un ácido graso C_{12} a C_{22}, o una mezcla de los mismos, dicho triglicérido, diglicérido o monoglicérido contiene residuos de ácido graso C_{12} a C_{22} unidos a al menos un grupo alcohol en la porción glicérica;

el surfactante principal comprende un aceite vegetal polioxietilen glicolado; y

el solvente miscible con agua farmacéuticamente aceptable es un derivado de α-hidro-ω -hidroxipoli(oxi-1,2-etanodiilo), etanol o alcohol bencílico.

15

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 16 - 0034930

Bogotá D.C., Abril 13 de 2016 - 11:09:08

RECIBIDO DE : CAVELIER ABOGADOS NI 860.041.367

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	706314464	13/04/2016	10.283.000.00

*** Conceptos Pagados ***

CANT.	RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1	50005-01-03 CAMBIO DE MODALIDAD Y MODIFICACIONES	EN N.CREACION CORRECCION A SOLICITUD EN TRAMITE / MODIFICACION CAP. DESCRIPTIVO O REIVINDICATORIO	164.000.00	164.000.00
				$164.000.00

SON: **CIENTO SESENTA Y CUATRO MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-*********-

Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 13-236457- -00011-0000
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Act: 449 RESPUESOPOSICI Folios: 47

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Durga Maheswari Parvataneni *et al*

Application No.: 14/003,445 (National stage of PCT/IB2011/001842) — Confirmation No.: 5330

Filed: August 10, 2011 — Art Unit: 1629

For: FORMULATION COMPRISING PHENYLAMINOPYRIMIDINE DERIVATIVE AS ACTIVE AGENT — Examiner: Lee, William Y

DECLARATION UNDER 37 C.F.R. § 1.132

I declare:

1. I am Senior Manager of Formulation Research and Development at Natco Pharma Limited.

2. I have completed a M. Pharmacy at the Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh and am currently pursuing a Ph.D. entitled "Formulations of poorly water soluble drugs using micellar solubilization technology" from Jawaharlal Nehru Technological University, Hyderabad, India. I have 15 years of experience in research activities, have published 4 research papers and have guided 18 students from M. Pharmacy for their research projects. I have worked as Senior Manager, Formulation Research & Development, Natco Pharma Limited, Hyderabad, India since May, 2003 and prior to this served as a chemist and Assistant Manager, R&D, at Tablets (India) Limited, Chennai, India.

3. I have read and understood (i) US patent application 14/003,445.

4. An experiment was performed to determine **the solubility of NRC-AN-019 in polar solvent (Water).** The procedure was as follows. Equilibrium solubility experiments were carried out in a 250 mL volumetric flask or conical flask, maintained at room temperature. A solid sample of known portion was transferred into a 250 mL conical flask containing 50 mL of polar solvent and shaken at 200 rpm on a rotary shaker. Aliquots were taken at regular intervals, filtered, diluted and injected into the HPLC system.

The Solubility Conditions were: Medium: Water; Volume: 50 mL; RPM : 200; Temperature: Room temperature; and Time points: About 1, 2, and 4 hours.

The Chromatographic Conditions were: Column: Purospher star RP 18e (150 x 4.6 mm) 5µ; Flow rate: 2.0 mL/min; Injection volume: 20 µL; Detection: 270 nm; Column Oven Temperature: 40°C; and Run Time: 14 minutes.

The Preparations were as follows.

Preparation of pH 3.0 Buffer solutions: Dissolved 1.36 g of potassium dihydrogen phosphate in 1000 mL of milli-Q water and adjusted pH to 3.0 with ortho phosphoric acid and filtered through 0.45 µ filter.

Preparation of Mobile phase: Mixed pH 3.0 buffer and acetonitrile in the ratio of 45:55 v/v and degassed for about 10 minutes.

Diluent: Mixed water and acetonitrile in the ratio of 5:96 v/v and degassed for about 10 minutes.

Standard preparation: Accurately weighed and transferred about 50 mg of NRC-AN-019 (WS) into a 100 mL volumetric flask, added 1mL of dimethylformamide, swirled and added about 70 mL of methanol, sonicated to dissolve, allow the solution to cool to room temperature and diluted to volume with methanol. Pipetted out 5 mL of the above solution

into a 50 mL volumetric flask and diluted to volume with diluent. Further diluted 2 mL of the above solution to 50 mL with diluents (2 ppm).

Test Preparation: Weighed and transferred accurately about 10 mg of NRC-AN-019 into a 250 mL conical flask, added 50 mL of medium, swirled and observed the solubility. If found as soluble, check added another 10 mg of NRC-AN-019, swirled, observed the solubility and repeated the procedure till some sample remains insoluble. Then shaken it on a rotary shaker for an hour and observed the solubility. If found soluble, added another portion (10 mg), shaken for an hour then repeated the procedure till some sample remains insoluble. Continue the shaking for 4 hours and collect 5 mL of sample solution at regular intervals like 1st Hr, 2nd Hr and 4th HR. The sample solution was filtered and discarded some portion, collected the remaining and diluted to a concentration which is in the linear range of standard concentration and injected into the HPLC system.

Result: The solubility of NRC-AN-019 in water: **no solubility even after 4th hour.**

5. An experiment was performed to determine **the solubility of NRC-AN-019 in non polar solvents (1-Octanol, n-Hexane, Toluene and cyclohexane).** The procedure was as follows. Equilibrium solubility experiments were carried out in a 250 mL volumetric flask or conical flask, maintained at room temperature. A solid sample of known portion was transferred into a 250 mL conical flask containing 50 mL of nonpolar solvent and shaken at 200 rpm on a rotary shaker. Aliquots were taken at regular intervals, filtered, diluted and injected into the HPLC system.

The Solubility Conditions were: Medium: 1-Octanol, n-Hexane, Toluene and cyclohexane; Volume: 50 mL; RPM : 200; Temperature: Room temperature; and Time points: About 1, 2, and 4 hours.

The Chromatographic Conditions were: Column: Purospher star RP 18e (150 x 4.6 mm) 5µ;

Flow rate: 2.0 mL/min; Injection volume: 20 µL; Detection: 270 nm; Column Oven Temperature: 40°C; and Run Time: 14 minutes.

The Preparations were as follows.

Preparation of pH 3.0 Buffer solutions: Dissolved 1.36 g of potassium dihydrogen phosphate in 1000 mL of milli-Q water and adjusted pH to 3.0 with ortho phosphoric acid and filtered through 0.45 µ filter.

Preparation of Mobile phase: Mixed pH 3.0 buffer and acetonitrile in the ratio of 45.55 v/v and degassed for about 10 minutes.

Diluent: Mixed water and acetonitrile in the ratio of 5:95 v/v and degassed for about 10 minutes.

Standard preparation: Accurately weighed and transferred about 50 mg of NRC-AN-019 (WS) into a 100 mL volumetric flask, added 1mL of dimethylformamide, swirled and added about 70 mL of methanol, sonicated to dissolve, allow the solution to cool to room temperature and diluted to volume with methanol. Pipetted out 5 mL of the above solution into a 50 mL volumetric flask and diluted to volume with diluent. Further diluted 2 mL of the above solution to 50 mL with diluents (2 ppm).

Test Preparation: Weighed and transferred accurately about 10 mg of NRC-AN-019 into a 250 mL conical flask, added 50 mL of medium, swirled and observed the solubility. If found as soluble, check added another 10 mg of NRC-AN-019, swirled, observed the solubility and repeated the procedure till some sample remains insoluble. Then shaken it on a rotary shaker for an hour and observed the solubility. If found soluble, added another portion (10 mg), shaken for an hour then repeated the procedure till some sample remains insoluble. Continue the shaking for 4 hours and collect 5 mL of sample solution at regular intervals like 1st Hr, 2nd Hr and 4th Hr. The sample solution was filtered and discarded some

portion, collected the remaining and diluted to a concentration which is in the linear range of standard concentration and injected into the HPLC system.

Result: The solubility of NRC-AN-019 in the tested non-polar solvents were as follows.

a) **1-Octanol : 535.48 µg/mL at 4th hour.**
b) **N-Hexane : 0.86 µg/mL at 4th hour.**
c) **Toluene : 13.20 µg/mL at 4th hour.**
d) **Cyclohexane : 0.22 µg/mL at 4th hour.**

6. An experiment was performed to determine **the solubility of NRC-AN-019 in different lipids.**

Note: As excipients are having solubility in different diluents, it was unable to fix a common diluent and chromatographic conditions. Hence, the solubility of NRC-AN-019 was determined in normal phase as well as in reverse phase conditions using different diluents and chromatographic conditions.

Normal phase Chromatographic Conditions: Column : Inertsil Silica (250 mm x 4.6 mm) 5 µ; Flow rate: 1.0 mL/min; Injection volume: 50 µL; Detection: 270 nm; Column temperature: 30 °C; and Run Time: 12 minutes.

Preparation of mobile phase: Mixed n-Hexane, Isopropyl alcohol and methanol in the ratio of 70:20:10 v/v respectively and degassed for about 5 minutes.

Standard preparation: Accurately weighed and transferred about 50 mg of NRC-AN-019 (WS) into a 100 mL volumetric flask and added about 70 mL of methanol, sonicated to dissolve, allowed the solution to cool to room temperature and diluted to volume with methanol. Transferred 4 mL of the above solution into a 100 mL and made up to volume with diluents (20 ppm).

Test Preparation: Weighed about 1200 mg of sample into an eppendorf tube and kept for centrifugation at 10000 rpm for 30 minutes. Then, weighed about 300 mg of centrifugate into a 50 mL volumetric flask and diluted to volume using a solvent or mixture, until clear solution is obtained. If required dilute suitably to a concentration, which is in the linear range of standard concentration and injected into the HPLC system.

Reverse phase Chromatographic conditions: Column: Purospher star RP 18e (150 x 4.6 mm) 5µ; Flow rate: 2.0 mL/min; Wave length: UV-270 nm; Column temperature: 40°C; Injection volume: 20 µL; and Run Time: 14 minutes

Buffer preparation: Weighed and dissolved about 1.36 g of Potassium dihydrogen phosphate in 1000 mL Milli Q water and adjusted the pH to 3.0 with orthophosphoric acid, filtered the buffer solution through 0.45 µ membrane filter.

Mobile phase preparation: Mixed pH 3.0 buffer, acetonitrile in the ratio of 45:55 v/v respectively and degassed the mixture for about 5 minutes.

Standard preparation: Accurately weighed and transferred about 100 mg of NRC-AN-019 working standard into 100 mL volumetric flask, added 1mL of dimethylformamide, swirled the contents to dissolve, added about 70 mL of methanol, sonicated, allowed the solution to cool to room temperature and diluted to volume with methanol. Transferred 4 mL of above solution into a 100 mL volumetric flask and diluted to volume with diluent (40 ppm).

Test preparation: Weighed about 1200 mg of sample into an eppendorf tube and kept for centrifugation at 10000 rpm for 30 minutes. Then, weighed about 300 mg of centrifugate into a 50 mL volumetric flask and diluted to volume using a solvent or mixture, until clear solution is obtained. If required dilute suitably to a concentration, which is in the linear range of standard concentration and injected into the HPLC system.

Procedure: Separately injected equal volumes (20 µL) of blank, standard preparation (six injections) and sample preparations into the chromatograph and recorded the chromatograms.

System suitability: Taken chromatograms of the standard preparation (six injections), measured the peak area for the analyte peak and evaluated the system suitability parameters. The system suitability parameters were met with the following acceptance criteria: (i) % RSD for six injections of peak area of NRC-AN-019 from the standard preparation should not be more than 2.0; (ii) tailing factor for NRC-AN-019 from the standard preparation should not be more than 2.0; and (iii) Plate count for NRC-AN-019 from the standard preparation should not be less than 2000.

Results:

S. No.	B. No & Exp No.	Sample code	Sample Name	Solubility (mg/mL)
1	FRD/171/029-01 & 019-LBF-040 (ADL/115)	API/L/01	NRC-AN-019 + Oleic acid	1.32
2		API/L/02	NRC-AN-019 + MCT	0.22
3		API/L/03	NRC-AN-019 + Olive oil	0.06
4		API/L/04	NRC-AN-019 + Sunflower oil	0.09
5		API/L/05	NRC-AN-019 + Castor oil	0.40
6		API/L/06	NRC-AN-019 + Peanut oil	0.12
7		API/L/07	NRC-AN-019 + Coconut oil	0.12
8		API/L/08	NRC-AN-019 + Soyabean oil	0.06
9		API/L/09	NRC-AN-019 + Palm oil	0.09
10		API/L/10	NRC-AN-019 + Sesame oil	0.12
11	FRD/171/030 & 019-LBF-041 (ADL/115)	API/L/11	NRC-AN-019 + Mustard oil	0.09
12	FRD/171/032 &		NRC-AN-019 + Lauroglycol	1.16
13			NRC-AN-019 + Labrafac	0.33

14	019-LBF-042 (ADL/115)		NRC-AN-019 + Labrafil	**0.63**
15			NRC-AN-019 + Peceol	**0.35**
16	FRD/171/037 & 019-LBF-048 (ADL/115)	API/L/68	NRC-AN-019 + Capmul MCM	**1.40**
17		API/L/70	NRC-AN-019 + Caprol 3 GO	**0.20**
18	FRD/171/037 & 019-LBF-048 (ADL/115)	API/L/72	NRC-AN-019 + Captex 355	**0.23**
19	FRD/171/037 & 019-LBF-048 (ADL/115)	API/L/74	NRC-AN-019 + Caprol 10 G 100	**0.20**
20		API/L/78	NRC-AN-019 + Canola oil	**0.06**
21	FRD/171/037 & 019-LBF-048 (ADL/115)	API/L/82	NRC-AN-019 + Almond oil	**0.07**
22	FRD/171/039-02 & 019-LBF-049 (ADL/115)	API/L/76	NRC-AN-019 + Caprol ET	**0.27**
23	FRD/194/057 & 019-LBF-063 (ADL/115)	API/L/101	NRC-AN-019 + Crodamol	**0.26**
24	FRD/194/067 & 019-LBF-075 (ADL/115)	API/L/147	NRC-AN-019 + Imwitor 988	**1.73**
25		API/L/148	NRC-AN-019 + Imwitor 742	**1.25**
26	FRD/196/102 & 019-LBF-091 (ADL/120)	API/L/289	NRC-AN-019 + Ethyl oleate	**0.14**
27	FRD/196/114 & 019-LBF-099 (ADL/120)	API/L/307	NRC-AN-019 + Oleyl alcohol	**0.44**
28	FRD/210/119 & 019-LBF-118 (ADL/128)	API/L/320	NRC-AN-019 + Corn oil, refined & pure	**0.09**
29		API/L/321	NRC-AN-019 + Labrafac lipofile WL 1349	**0.23**
30		API/L/322	NRC-AN-019 + Lauroglycol FCC	**0.60**
31		API/L/328	NRC-AN-019 + Capmul GMO – 50	**0.30**
32		API/L/395	NRC-AN-019 + Labrafil M 1944 CS	**0.53**
33		API/L/396	NRC-AN-019 + Plurol Olique CC	**0.29**

Application No.: 14/003,445

	FRD/228/173 & 019-LBF-234 (ADL/135)		497	
34		API/L/397	NRC-AN-019 + Labrafil M 2125 CS	**0.51**
35	FRD/239/219 & AR/006/11/2010 (ADL/149)	API/L/412	NRC-AN-019 + Caprol PGE 860	**0.24**
36	FRD/244/250-02 & AR/034/12/2010 (ADL/150)	API/L/413	NRC-AN-019 + Ricinoleic acid	**3.24**
37		API/L/416	NRC-AN-019 + Linseed Oil	**0.09**
38	FRD/245/274 & AR/127/01/2011 (ADL/158)	API/L/423	NRC-AN-019 + Captex 200 P	**0.28**
39		API/L/424	NRC-AN-019 + Caproyl 90	**2.19**
40		API/L/429	NRC-AN-019 + Caprol MPGO	**0.65**
41		API/L/431	NRC-AN-019 + Liquid paraffin	**0.17**
42		API/L/432	NRC-AN-019 + Corn Oil Sunflower Oil blend	**0.19**
43	FRD/246/295-02 & AR/056/02/2011 (ADL/159)	API/L/441	NRC-AN-019 + Linseed Oil	**0.10**
44		API/L/442	NRC-AN-019 + Linoleic acid	**2.43**
45	FRD/246/313 & AR/047/04/2011 (ADL/161)	API/L/449	NRC-AN-019 +Captex 355	**0.23**
46		API/L/451	NRC-AN-019 + Miglyol 810	**0.22**
47		API/L/455	NRC-AN-019 + Lipoid MCT	**0.22**
48		API/L/457	NRC-AN-019 + Crodamol GTCC	**0.22**
49		API/L/463	NRC-AN-019 + Labrafac lipofile WL 1349	**0.24**
50		API/L/465	NRC-AN-019 + Capmul MCM	**1.19**
51	FRD/246/327 & AR/086/04/2011 (ADL/162)	API/L/453	NRC-AN-019 + Miglyol 812 N	**0.23**
52		API/L/473	NRC-AN-019 + Labrafil 1944 CS	**0.60**
53		API/L/475	NRC-AN-019 + Labrafil 2125	**0.59**
54		API/L/477	NRC-AN-019 + Captex 300	**0.24**
55		API/L/483	NRC-AN-019 + Linoleic acid	**3.27**
56	FRD/257/347 & AR/021/05/2011 (ADL/162)	API/L/489	NRC-AN-019 + Cetiol LC PH	**0.14**
57		API/L/491	NRC-AN-019 + Cetiol V PH	**0.10**
58	FRD/258/372 & AR/035/06/2011	API/L/507	NRC-AN-019 + Cod liver oil	**0.06**

Application No.: 14/003,445

	(ADL/165)			
59	FRD/258/378 & AR/069/06/2011 (ADL/165)	API/L/513	NRC-AN-019 + Incromega	**0.12**
60	FRD/276/465 & AR/116/08/2011 (ADL/174)	API/L/548	NRC-AN-019 + Ethyl Oleate	**0.13**
61	FRD/276/466 & AR/023/09/2011 (ADL/174)	API/L/555	NRC-AN-019 + Caproyl-90	**1.98**
62		API/L/556	NRC-AN-019 + Lauroglycol FCC	**0.54**
63	FRD/277/469 & AR/038/09/2011 (ADL/174)	API/L/562	NRC-AN-019 + Capmul PG-8	**1.85**
64		API/L/563	NRC-AN-019 + Lauroglycol-90	**0.81**
65		API/L/564	NRC-AN-019 + Caproyl PGMC	**1.67**
66		API/L/565	NRC-AN-019 + Capmul GMO	**0.34**
67		API/L/566	NRC-AN-019 + Peceol	**0.35**
68		API/L/567	NRC-AN-019 + Captex 200P	**0.24**
69	FRD/277/472 & AR/062/09/2011 (ADL/174)	API/L/574	NRC-AN-019 + Caprol 3GO	**0.31**
70		API/L/575	NRC-AN-019 + Caprol MPGO	**0.44**
71		API/L/576	NRC-AN-019 + Capmul MCM	**1.19**
72		API/L/577	NRC-AN-019 + Imwitor-742	**1.30**
73		API/L/578	NRC-AN-019 + Caprol PEG-860	**1.78**
74		API/L/579	NRC-AN-019 + Capmul 708G	**0.99**
75	FRD/277/474 & AR/086/09/2011 (ADL/174)	API/L/586	NRC-AN-019 + Maisine 35-1	**0.33**

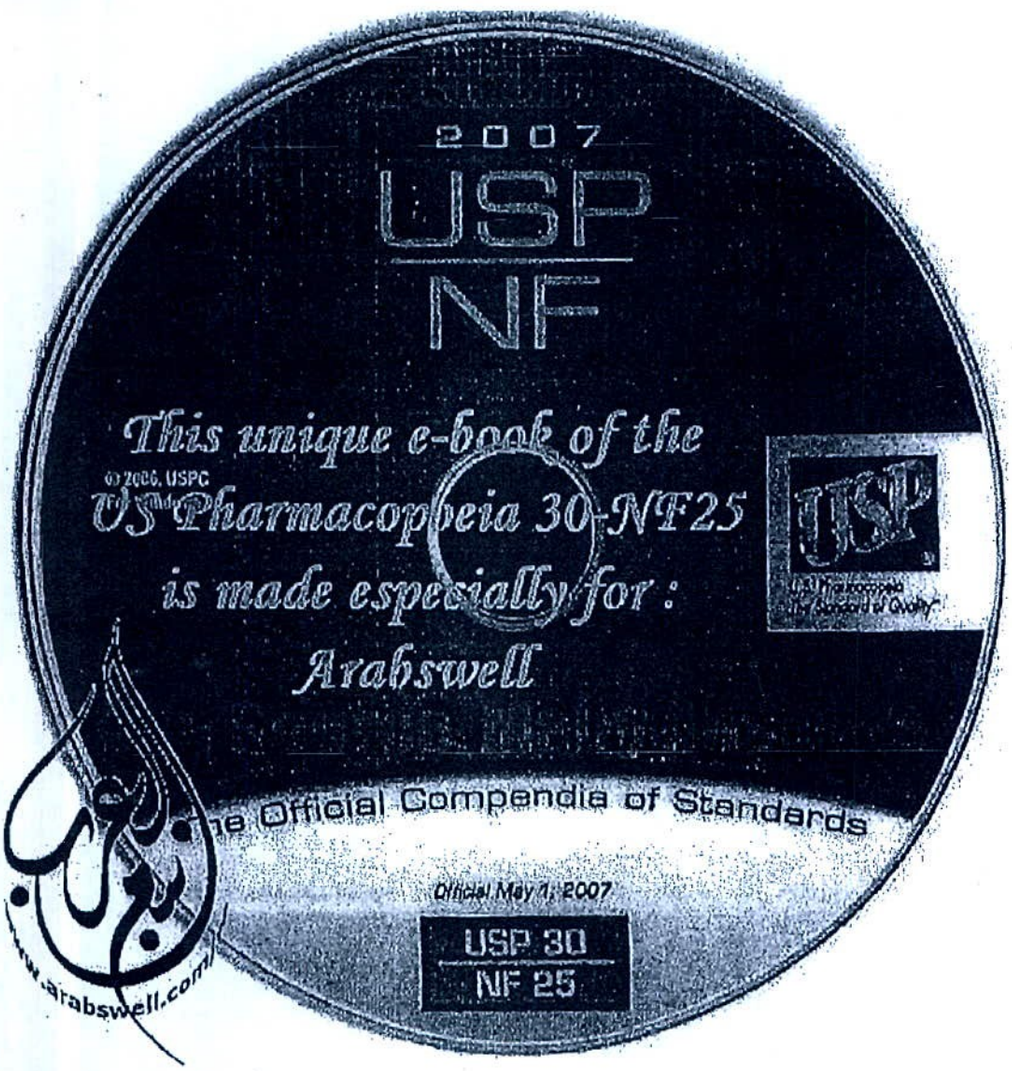
Date: 22.10.2014

Durga Maheswari. P

Durga Maheswari Parvataneni

Annexure : 4

Introduction



Best Regards,

Lawrence

DESCRIPTION AND SOLUBILITY

Description and Relative Solubility of USP and NF Articles

The "description" and "solubility" statements pertaining to an article (formerly included in the individual monograph) are general in nature. The information is provided for those who use, prepare, and dispense drugs, solely to indicate descriptive and solubility properties of an article complying with monograph standards. The properties are not in themselves standards or tests for purity even though they may indirectly assist in the preliminary evaluation of the integrity of an article.

Taste and Odor

Organoleptic characteristics are indicated in many instances because they may be useful and descriptive properties of substances. However, they are not meant to be applied as tests for identifying materials.

The inclusion of odor or taste among other descriptive properties may aid in identifying the causative agent following accidental exposure to or contact with a substance. This information is provided as a warning or to make an individual aware of sensations that may be encountered. The use of odor or taste as a test for identification or content is strongly discouraged.

The characteristic odor of a volatile substance becomes apparent immediately on opening a container of it. The odor may be agreeable (e.g., Peppermint Oil), unpleasant (e.g., Sulfur Dioxide), or potentially hazardous on prolonged exposure (e.g., Coal Tar). Moreover, an unexpected odor may be encountered if the characteristics of a substance are not known or if a container is incorrectly labeled. Consequently, containers of such substances should be opened cautiously, preferably in a well-ventilated fume hood. A characteristic taste or sensation produced in the oral cavity likewise is apparent if traces of residue materials on fingers are inadvertently brought into contact with the tongue or adjacent mucosal tissues.

Solubility

Only where a special, quantitative solubility test is given in the individual monograph, and is designated by a test heading, is it a test for purity.

The approximate solubilities of Pharmacopeial and National Formulary substances are indicated by the descriptive terms in the accompanying table. The term "miscible" as used in this Pharmacopeia pertains to a substance that yields a homogeneous mixture when mixed in any proportion with the designated solvent.

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble, or Insoluble	10,000 and over

Soluble Pharmacopeial and National Formulary articles, when brought into solution, may show traces of physical impurities, such as minute fragments of filter paper, fibers, and other particulate matter, unless limited or excluded by definite tests or other specifications in the individual monographs.

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Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs

Christopher J. H. Porter, Natalie L. Trevaskis and William N. Charman

Abstract | Highly potent, but poorly water-soluble, drug candidates are common outcomes of contemporary drug discovery programmes and present a number of challenges to drug development — most notably, the issue of reduced systemic exposure after oral administration. However, it is increasingly apparent that formulations containing natural and/or synthetic lipids present a viable means for enhancing the oral bioavailability of some poorly water-soluble, highly lipophilic drugs. This Review details the mechanisms by which lipids and lipidic excipients affect the oral absorption of lipophilic drugs and provides a perspective on the possible future applications of lipid-based delivery systems. Particular emphasis has been placed on the capacity of lipids to enhance drug solubilization in the intestinal milieu, recruit intestinal lymphatic drug transport (and thereby reduce first-pass drug metabolism) and alter enterocyte-based drug transport and disposition.

Enterocyte
The absorptive cells lining the small intestine.

Dissolution rate
The rate at which a solute (for example, a drug) dissolves in a solvent.

Polymorphs
A specific crystalline form of a compound (for example, a drug) that exhibits polymorphism, that is the ability to crystallize in different forms.

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Lipids and lipophilic excipients can have significant and beneficial effects on the absorption and exposure of co-administered lipophilic drugs. Typically, these effects have been documented as a result of empirical investigations of the performance of pharmaceutical formulations, and a rational basis for lipid and lipophilic excipient selection remains elusive. The corresponding poor prediction of *in vivo* performance has therefore limited the widespread adoption of lipid-based strategies for enhancing drug exposure. In terms of drug discovery and development, however, there is a prescient requirement for the identification of robust and effective means of enhancing the bioavailability of poorly water-soluble drugs. This is especially the case when potent, but highly lipophilic, drug candidates arise from complex chemical scaffolds and multiple high-throughput activity screens[1].

There are three primary mechanisms by which lipids and lipophilic excipients affect drug absorption, bioavailability and disposition after oral administration. These are the alteration of the composition and character of the intestinal milieu, the recruitment of intestinal lymphatic drug transport, and the interaction with enterocyte-based transport processes (FIG. 1). Here we discuss and evaluate the opportunities associated with each scenario.

Intestinal drug solubilization

With few exceptions, molecular dispersion of a drug is a prerequisite for its absorption across biological membranes. After oral administration, this dictates that the drug must first dissolve within the gastrointestinal (GI) tract before partitioning into and then across the enterocyte. The absorption of poorly water-soluble drugs can be limited by the dissolution rate and the extent to which the drug dissolves. The thermodynamic principles governing drug solubilization indicate that improved solubility is favoured by reduced intermolecular forces in the solid state and enhanced solute–solvent interactions in the bulk solution. Strategies to improve drug solubility by the alteration of solid-state properties include identification of advantageous polymorphs, hydrates or salts. The rate of drug dissolution can be enhanced through judicious manipulation of particle size and the generation of, for example, solid dispersion formulations. The primary mechanisms by which lipid-based drug formulations enhance drug solubilization within the GI tract are by presentation as a solubilized formulation (thereby avoiding solid-state limitations) and by induced changes to the character of the GI environment such that solute–solvent interactions and drug solubility are enhanced.

The nature of the GI fluids and their associated solubilization capacity can be regarded as the combined

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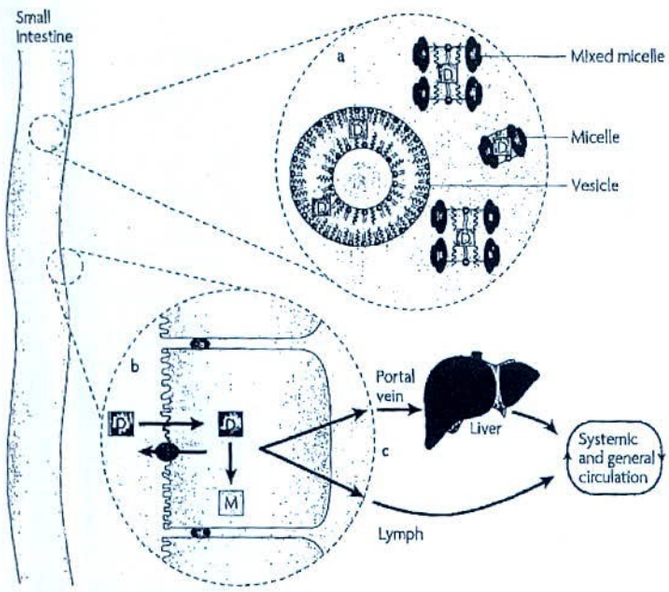


Figure 1 | **Potential effect of lipids and lipidic excipients on drug absorption.** Lipids can affect drug absorption in three ways: by enhancing drug (D) solubilization in the intestinal milieu through alterations to the composition and character of the colloidal environment — for example, vesicles, mixed micelles and micelles (**a**); by interacting with enterocyte-based transport and metabolic processes, thereby potentially changing drug uptake, efflux, disposition and the formation of metabolites (M) within the enterocyte (**b**); or by altering the pathway (portal vein versus intestinal lymphatic system) of drug transport to the systemic circulation — which in turn can reduce first-pass drug metabolism as intestinal lymph travels directly to the systemic circulation without first passing through the liver (**c**). Cellular junctions are represented by green ovals, and a representative transport protein is depicted by a blue oval.

effects of the intrinsic aqueous solubility of the drug, the enhancements in solubility resulting from the presence of endogenous solubilizing components, and the enhancements in solubility resulting from the presence of exogenous components (that is, formulation-derived). It is self-evident that certain exogenous components might be expected to lead to changes in the nature of the GI fluids and enhance drug solubilization. Typical examples of such components include surfactants, co-solvents and complexation agents. However, formulation- or food-derived lipids can influence GI solubilization through an increase in solubilization capacity attributable to the lipid itself and through stimulation of physiological processes, which lead to the enhanced secretion of endogenous biliary-derived solubilizing components such as bile salts and phospholipids. Examples of experimental values of bile salt and phospholipid concentrations in fasted and post-prandial human intestine are given in TABLES 1,2, respectively. The solubilization capacity of the GI tract is therefore determined by the interaction of exogenous lipids with the GI environment, the physiological changes that the lipid component stimulates and the combined involvement of both exogenous and endogenous components in the colloidal species that support enhanced drug solubilization. Cognizance of the events induced and stimulated by the presence of lipids in the GI tract is therefore essential to an understanding of the role of lipids as formulation excipients.

Solid dispersion
A solid-dose formulation that comprises a molecular mixture of a drug and a highly water-soluble excipient (commonly polyethylene glycol or polyvinylpyrrolidone).

Post-prandial
After a meal.

Post-prandial response
The physiological response that occurs after ingestion of a meal (in particular, a fatty meal) including delayed gastric emptying, release of bile and pancreatic secretions, and alterations in gastrointestinal motility and secretions.

Lipids and the GI environment

Much of the knowledge base describing the digestion and absorption of lipids, and the resulting effect on GI physiology, has been derived from the study of post-prandial events. Unfortunately, these observations have largely been extrapolated to estimate the likely effect of formulation-related lipids on GI function — despite pharmaceutically relevant volumes of formulation lipid being typically less than 2–3 g. Persson *et al.* recently suggested that a post-prandial response is stimulated, at least in part, by as little as 7 g of lipid[2] and studies have shown similar drug absorption patterns after administration of 2–10 g of formulated lipid when compared with post-prandial administration[3–5]. Although the mechanisms that underlie the ability of lipid-based formulations to enhance bioavailability to post-prandial levels are complex, these data suggest that lipid-based formulations have the capacity to at least partially stimulate the 'lipid-sensing' mechanisms that promote the physiological changes that can assist drug absorption. Future studies will be required to evaluate the lipid-dose dependency of such induced physiological changes.

The absorption of lipophilic drugs from lipid-based formulations is determined by the patterns of dispersion, digestion and solubilization of formulation-derived lipid and co-administered drug in the GI tract (FIG. 2). After oral administration, the presence of lipid in the GI tract leads to secretion of gastric lipase from the chief cells lining the gastric mucosa[6,7] and secretion of pancreatic lipase and co-lipase from the pancreas[8]. Gastric lipase initiates lipid digestion in the stomach, which results in the partial digestion of triglyceride to diglyceride and fatty acid[9,10]. Although gastric lipolysis is a minor contributor to the overall lipid digestion process, it has been suggested to be responsible for up to 25% of acyl chain hydrolysis[11,12], indicating that some gastric processing of lipidic formulations is likely. The magnitude of any effect will depend on the residence time of the formulation within the stomach and its dispersion properties and intrinsic susceptibility to digestion. Lipids are crudely emulsified in the stomach, and subsequently enter the small intestine where quantitative digestion of triglyceride is completed by pancreatic lipase at the oil–water interface[13]. The presence of lipids and lipid-digestion products in the GI tract stimulates secretion of bile into the small intestine from the gall bladder. The components of bile[14] provide a vehicle for the solubilization of the poorly water-soluble fatty acid, monoglyceride and diglyceride products of lipid digestion, which are incorporated into a series of colloidal structures (FIG. 2). The presence of lipid and the resulting digestion products in the small intestine also slows the rate of delivery of material from the stomach, presumably to enable the more effective digestion and absorption of lipids in the upper GI tract. The specific mechanisms by which lipids reduce gastric emptying are not clear

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Table 1 | **Fasting intestinal bile salt and phospholipid concentrations**

Fasted bile salt concentration (mM)	Fasted phospholid concentration (mM)	Sample location	Refs
2 ± 0.2 (mean ± SD, n = 3)	0.2 ± 0.07	Jejenum	28
2.82 (pooled, n = 12)	NR	Duodenum	208
2.0 ± 1.9 (mean ± SD, n = 9)	NR	Jejenum	209
2.9 ± 2.9 (mean ± SD, n = 37)	NR	Jejenum	211
5.9 ± 1.8 (mean ± SE, n = 7)	NR	Duodenum	210
5.3 ± 4.7* (mean ± SD, n = 16)	NR	Jejenum	213
4.4 ± 1.8* (mean ± SD, n = 20)	NR	Jejenum	214
6.4 ± 1.3‡ (mean ± SE, n = 7)	NR	Duodenum	215

*Concentrations estimated from graphical representations. ‡Concentrations attained during periods preceding a migrating motor complex. NR, not reported.

and competing factors such as osmolality, calorific volume and pH are also likely to mediate changes to the gastric-emptying patterns[15–17]. A number of secondary biochemical mediators such as cholecystokinin[17,18], chylous lymph and apolipoprotein A-IV (REFS 19–21) have also been implicated in lipid-induced effects on satiety and gastric motility. Although the biochemical mechanism by which lipids affect GI motility remains the subject of debate, most studies suggest that the initial stimulatory event is the presence of lipid in the small intestine and that long-chain (rather than medium-chain) fatty acids seem to be most effective[17,22–24].

A further barrier to the effective uptake of lipids from the intestinal lumen into enterocytes is diffusion across the unstirred water layer, which separates the bulk fluid phase of the small intestine lumen from the brush border membrane of enterocytes[25,26] (BOX 1). Solubilization of fatty acids and monoglycerides (and lipophilic drugs) in micellar and mixed-micellar structures, however, can greatly enhance the mass transport of molecules across the unstirred water layer, thereby enhancing lipid and drug absorption.

The colloidal structures formed during the digestion of lipids provide a series of enduring lipophilic phases within which lipophilic drugs might reside during GI transit, thereby preventing precipitation and enhancing absorption of the drugs. The nature of the colloidal species formed by the intercalation of formulation components and their digestion products with endogenous biliary-secreted bile salt, phospholipid and cholesterol species is therefore a crucial determinant of the corresponding patterns of drug solubilization and drug absorption and is described in detail below.

Bile
A fluid secreted from hepatocytes in the liver and stored in the gall bladder before release into the small intestine. The primary constituents of bile are water, bile salt, cholesterol, phospholipid, bicarbonate, bile pigments and organic wastes. Bile salt, cholesterol and phospholipid are co-secreted in bile in the form of mixed micellar complexes in a molar ratio of approximately 1:6:4:1.

Critical micelle concentration
The minimum concentration of a surfactant in a bulk solution that leads to spontaneous formation of surfactant micelles. Also, the concentration of free surfactant in solution that is in equilibrium with surfactants in a micellar (aggregated) form.

Exogenous and endogenous solubilizing species

Under fasted conditions, the solubilizing species present in the intestinal contents comprise low concentrations of bile salt, phospholipid and cholesterol derived from fasted biliary output[2,27–29]. In the presence of phospholipid, bile salt concentrations remain above the critical micelle concentration; however, the nature and size of the colloidal structures present are dependent on the proportions of each of the included components[30,31]. Nevertheless, in the absence of exogenous lipids the solubilization capacity of the fasted small intestine remains low and is correlated with total bile salt concentrations rather than reflecting the structure of the individual colloidal species present[32,33].

Conversely, following addition of lipids that are representative of the digestion products of exogenously derived (from formulation or food) lipids, the drug solubilization capacity increases significantly and is dependent on the nature of the digestion products (in terms of fatty-acid chain length) and the characteristics of the colloidal structures that they form. For example, the digestion products of medium-chain triglycerides (C_{8-12} fatty acids and monoglycerides) are amphiphilic and readily combine with endogenous bile salt, phospholipid and cholesterol to provide highly dispersed, optically clear dispersions (even at high (~150 mM) lipid loads). The drug solubilization capacity of these composite colloidal species can be up to 50-fold higher than that of endogenous bile salt, phospholipid and cholesterol species[30]. However the solubilization capacity is dependent on lipid concentration, and the solubility of a range of poorly water-soluble drugs has been shown to be enhanced by less than threefold at lower (<25 mM) exogenous lipid levels[30,34]. By contrast, the phase behaviour and solubilization characteristics of the species formed on intercalation of the digestion products of long-chain triglyceride (which comprises primarily C_{18} lipids) vary significantly when compared to medium-chain triglycerides[34] (BOX 2). C_{18} fatty acids and monoglycerides are considerably less polar than their C_8 or C_{12} equivalents and turbid systems that contain larger (~100 nm) vesicular species are evident even at low (>2.5 mM) lipid concentrations. Importantly, these vesicular species provide for significantly enhanced drug solubilization capacities. For example, in the presence of 8.75 mM long-chain fatty acids and 4.4 mM long-chain monoglycerides (approximately the same mass per mass quantities of lipid that led to a less than a threefold improvement in solubilization capacity for the medium-chain lipids) solubilization enhancements of up to 20-fold are apparent[30,34]. These solubilization differences, which are based on lipid content, are particularly significant in the context of the likely luminal concentration of lipid obtained after oral administration of a lipid-based formulation. For example, assuming a luminal volume of 200 ml, a lipid dose of 750 mg long-chain triglyceride and complete digestion, the maximal luminal concentrations of fatty acid and monoglyceride (solubilized in micelles) post-digestion are approximately 8.5 mM and 4.2 mM, respectively.

Assessment of lipid-based formulations

The realization that the performance of lipid-based formulations is affected by digestion and the incorporation of exogenous digestion products into endogenous micellar species has led to the increasingly widespread use of lipid digestion models for *in vitro* assessment of lipid-based formulations[27,35–38,243] (FIG. 3). Dynamic lipolysis experiments have enhanced the understanding of the changes to solubilization capacity that might occur

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Table 2 | **Summary of literature values for post-prandial concentrations of bile salt and phospholipid in the human small intestine**

Fed bile salt concentration (mM)	Fed phospholipid concentration (mM)	Time post-prandial	Meal	Sample location	Refs
8 ± 0.1 (mean ± SD, n = 3)	3 ± 0.3	20–60 min after start of perfusion	NuTRIflex nutritional drink containing 7.2 g lipid perfused into jejunum over 90 min	Jejunum	28
11.8 (pooled, n = 12)	4.31 (pooled, n = 12)	1 h	500 ml of Ensure Plus	Duodenum	208
10.1 ± 4.2 (mean ± SE, n = 7)	6.3 ± 1.0	1 h	70 g olive oil, 1 egg, 1 egg white and 70 g sucrose in 400 ml water	Duodenum	210
14.5 ± 8.8 (mean ± SD, n = 5)	4.8 ± 1.8	1–2 h	50 g olive oil, 1 egg, 2.14 g NaCl (0.09 M) and 20 g sucrose in 400 ml water	Duodenum	212
14.7 ± 8.0 (mean ± SD, n = 16)	NR	0.5 h	15 g maize oil, 30 g skimmed milk powder, 35 g glucose in 200 ml water	Jejenum	213
15.8 ± 5.6* (mean ± SD, n = 5)	NR	0.5–1 h	Meal supplemented with long-chain triglyceride, 48% carbohydrate, 35% fat, 16% protein, total 400 Kcal	Jejenum	214
6.8 ± 1.72* (mean ± SD, n = 5)	NR	0.5–1 h	Meal supplemented with medium-chain triglyceride, 48% carbohydrate, 35% fat, 16% protein, total 400 Kcal	Jejenum	214
16.2 ± 1.5 (mean ± SE, n = 13)	NR	0–0.5 h	30 g corn oil, 25 g Hyperprotidine water to 400 g	Jejenum	216
14.5 ± 9.4 5.2 ± 2.3 (mean ± SD, n = 12)	NR	0.5 h 1 h	NR	Duodenum	217

*Concentrations estimated from graphical representations. NR, Not reported.

on digestion of both lipids and surfactants that are commonly included in lipid-based formulations. These data have shown, for example, that drug solubilization in the presence of digesting medium-chain lipids is most efficient at high lipid loads[36], but is significantly reduced as the mass of exogenous lipid in the digest is decreased[33], and that digestion of certain surfactants might lead to significant decreases in solubilization capacity for some formulations[39]. By contrast, in the presence of long-chain triglycerides, effective drug solubilization is often possible even at low lipid concentrations[35]. These data suggest that in certain circumstances the use of medium-chain lipids in lipid-based formulations (even in the presence of surfactant) can lead to drug precipitation, and therefore reduced absorption, as a result of luminal digestion of the formulation lipid[3,35,38,40].

Although the data from our laboratories (and others), which describe trends in solubilization and oral bioavailability for highly lipophilic drugs such as halofantrine, cinnarizine[33] and cyclosporine[41], suggest the relative benefits of long-chain rather than medium-chain lipids[33,36,38], these effects are drug specific. There are also reports of enhanced oral bioavailability after administration in medium-chain rather than long-chain lipid-based vehicles for drugs such as progesterone and penclomedine[37,42]. However, even in the case of progesterone, a correlation between the solubilization profiles observed during *in vitro* lipolysis and oral bioavailability was still evident. Therefore, although preferential solubilization will be dictated by the physicochemical properties of individual drugs and their specific interactions with solubilizing colloidal structures, *in vitro* lipid digestion models seem to provide useful initial guidance with respect to formulation design.

First-pass metabolism
Drugs administered orally are typically taken up into the enterocytes lining the upper small intestine and transported by the mesenteric vessels to the hepatic portal vein and then to the liver before reaching the systemic circulation. First-pass metabolism refers to the metabolism of a drug within the liver and enterocytes before the drug first reaches the systemic circulation.

An additional feature to consider when comparing *in vitro* solubilization and *in vivo* bioavailability data, and in particular the impact of medium-chain or long-chain lipids, are the differential effects of these lipids on intestinal lymphatic transport[37,43]. These aspects are described later in more detail. However, it is important to note that medium-chain lipids are poor stimulators of intestinal lymphatic transport. If lymphatic transport of a drug is likely to provide a significant advantage in terms of bioavailability (for example, where hepatic first-pass metabolism is significant), it is therefore imprudent to attempt a correlation between solubilization and drug bioavailability.

In recent years, there has been increasing interest in the use of formulations that comprise combinations of natural lipids with surfactants, co-surfactants and co-solvents (see BOX 3 for details). There have been two drivers for investigating these more complex systems. First, the solubility of poorly water-soluble drugs in triglyceride lipids (and in particular long-chain triglycerides) is often low, thereby limiting the drug loading capacity of solution-based formulations. By contrast, drug solubility in amphiphilic surfactant and co-solvent systems is typically higher and this affords a strategy for increasing the maximum unit dose. Second, several studies have suggested that highly dispersed formulations — and in particular those that self emulsify on contact with GI fluids to provide submicron sized particles ('self-microemulsifying' formulations) — provide for enhanced bioavailability (reviewed in REFS 44,45). Although a detailed description of these formulation approaches is beyond the scope of this current discussion, the basic principles that dictate formulation performance in terms of GI solubilization remain unchanged. Therefore, regardless of the physical

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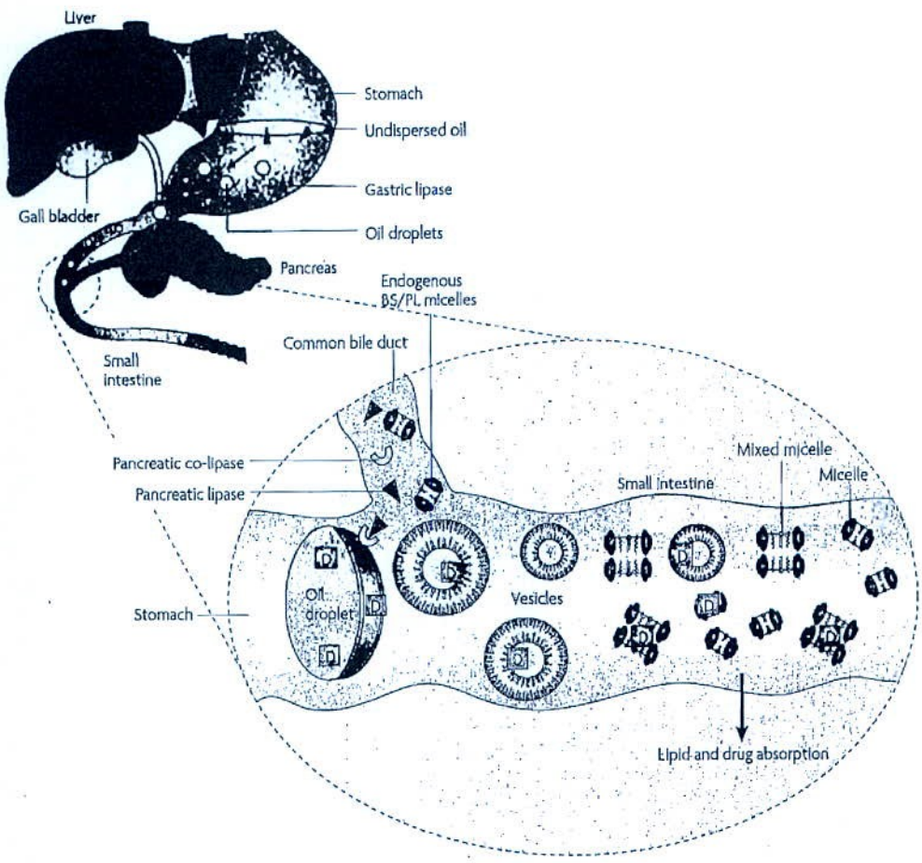


Figure 2 | **Lipid digestion and drug solubilization in the small intestine.** Following ingestion, the digestion of exogenous dietary triglyceride (TG) and formulation TG is initiated in the stomach by gastric lipase. The stomach further contributes to lipid processing by mechanical mixing (propulsion, grinding and retropulsion), which when combined with the presence of the amphiphilic products of initial lipid digestion (diglyceride and fatty acid) facilitates formation of a crude emulsion (lipid digestion by lingual lipase in the mouth might precede gastric digestion; however, pharmaceutical formulations are typically encapsulated so that their contents are released in the stomach after ingestion). In the small intestine, pancreatic lipase together with its cofactor co-lipase[203] completes the breakdown of TG to diglyceride, monoglyceride and fatty acid. Pancreatic lipase acts primarily at the sn-1 and sn-3 positions of TG to produce 2-monoglyceride and free fatty acid[203,204]. The chemical digestion of formulation- or biliary-derived phospholipid (PL) also occurs in the small intestine in which pancreatic phospholipase A_2 hydrolyses a single fatty-acid molecule from the sn-2 position of PL to yield lysophosphatidylcholine and fatty acid[205,206]. The presence of exogenous lipids in the small intestine also stimulates secretion of endogenous biliary lipids, including bile salt (BS), PL and cholesterol from the gall bladder. In the presence of raised BS concentrations, the products of lipid digestion (monoglyceride, fatty acid and lysophospholipid) are subsequently incorporated into a series of colloidal structures, including multilamellar and unilamellar vesicles, mixed micelles and micelles. Together these species significantly expand the solubilization capacity of the small intestine for lipid digestion products and drugs (D). The oil droplet in the intestine is stylistically represented in different colours to indicate undigested TG in the core (orange) and digested products such as fatty acid (blue) and monoglyceride (green) on the surface of the droplet.

form of the lipidic formulation that is introduced into the GI tract, or indeed the form it takes on initial dispersion in the GI fluids, the key issue is whether precipitation of a co-formulated drug is prevented as the formulation interacts with the GI environment. These interactions include the potential ability of lipids or other excipients to stimulate secretion of biliary lipids and alter gastric transit, the potentially significant changes to formulation properties that might occur on digestion and interaction with the bile salt, phospholipid and cholesterol micellar species in the GI tract, and the formation of a series of solubilizing lipidic microenvironments (emulsified, micellar, liquid crystalline or vesicular) derived from endogenous and exogenous sources.

As the initial physicochemical and colloidal properties of a lipidic formulation might be expected to persist for only a limited period *in vivo*, it is the colloidal species that form after interaction of the formulation with the GI environment that are the actual 'carriers' or solubilizing species for co-formulated poorly water-soluble drugs.

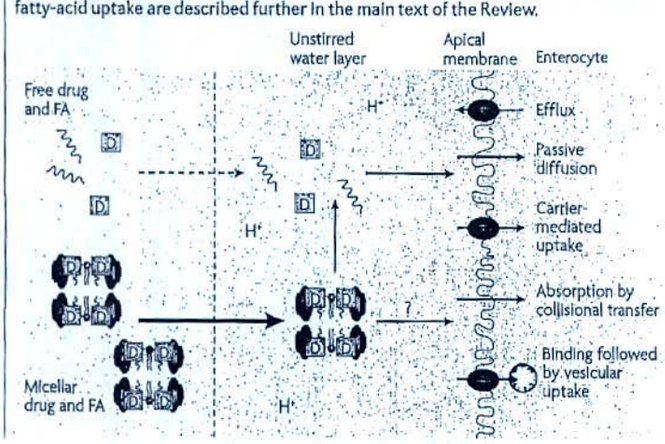
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Lipoproteins
Colloidal particles synthesized in the liver and small intestine that consist of a hydrophobic core (containing triglyceride and cholesteryl esters) and a hydrophilic surface (containing phospholipids, cholesterol and apolipoproteins). Lipoproteins facilitate the transport of lipids and lipophilic substances around the body.

Applying this premise, complex formulations that contain lipids, surfactants and co-solvents can also be assessed using *in vitro* lipolysis to provide an indication of performance, and this could gauge utility more robustly when compared with the historical metrics of solubility and particle size. Indeed recent data from our laboratories suggest that product performance is poorly related to the initial particle size of a dispersed formulation and instead is correlated more with the patterns of solubilization obtained after dispersion and digestion of the formulation in simulated intestinal fluid[3,46]. Parenthetically, it is self-evident that where an excipient effect on transporter function is suspected, and where permeability rather than solubility limits bioavailability, then measures of *in vitro* solubilization would be expected to poorly reflect trends in *in vivo* bioavailability.

Box 1 | The unstirred water layer as a barrier to lipid and drug absorption

The brush border (apical) membrane of enterocytes (see figure) is separated from the bulk fluid phase of the small intestine lumen by an unstirred water layer (UWL)[28]. The UWL mixes poorly with the bulk fluid phase and together with intestinal mucus forms an acidic microclimate (represented by H^+ ions on the diagram) adjacent to the brush border membrane. It is believed that the Na^+/H^+ and $Cl^-/HCO3^-$ exchange transporters contribute to this acidic microclimate[28]. Solute molecules in the bulk phase of the intestinal lumen must cross the UWL to gain access to the brush border membrane. This represents a major diffusional barrier for lipids and lipophilic molecules as their solubility in aqueous media is extremely low. As such, free fatty-acid (FA), monoglyceride (MG) and lipophilic molecules, such as lipophilic drugs (represented by D on the diagram), diffuse slowly across the UWL. However, micellar solubilization of FA, MG and lipophilic molecules greatly enhances their solubility in the UWL and, despite the slower diffusion rate of micelles across the UWL relative to single molecules (owing to size), micellar solubilization greatly enhances the mass transport of FA, MG[218] and lipophilic molecules[219] across the UWL. In the small intestine, micelles are not believed to be absorbed intact across the brush border membrane[220,221]. However, vesicular-mediated uptake of fatty acids has recently been demonstrated in HepG2 (human Caucasian hepatocyte carcinoma) cells, adipocytes and human microvascular endothelial cells[222–224,245], and such a mechanism can not, as yet, be entirely refuted for the small intestine. Several possible mechanisms for FA, MG and lipophilic drug uptake across the brush border membrane[220,238] are depicted in the figure below. FA, MG and lipophilic molecules may dissociate from the mixed micellar phase before partitioning into the enterocyte and in the case of FA it has been suggested that the acidic microclimate might facilitate micellar dissociation[225,226]. Free FA, MG and lipophilic drug molecules can then be absorbed across the apical membrane by passive diffusive diffusion or carrier-mediated transport. Free FA and drug molecules might also be effluxed back into the intestinal lumen by an efflux transporter. Alternatively, transfer from micelles to the brush border membrane may occur directly by a collisional mechanism (which could be facilitated by a carrier) or micelles might undergo vesicular-mediated uptake and this process could be initiated by the micelle binding to a transport protein on the apical membrane. Aspects of passive and carrier-mediated fatty-acid uptake are described further in the main text of the Review.



Intestinal lymphatic drug transport

The intestinal lymphatic system (BOX 4) is a unique, high-capacity drug transport pathway, which has the potential to enhance the exposure of co-administered lipophilic drugs. Currently, only a few drugs that are in clinical use are substantially transported to the systemic circulation by the intestinal lymphatic system following oral administration in their approved dosage form. However, the trend towards the identification of more potent and often more lipophilic development candidates is increasingly suggesting the progression of molecules with physicochemical characteristics that are more amenable to access to the intestinal lymphatic system. The key to exploiting the systemic exposure and drug delivery advantages associated with lymphatic transport are the prudent design of lipid-based formulations and the selection of relevant drug candidates. These advances are predicated on a better understanding of enterocyte biology and the mechanisms involved in the lymph–portal partitioning of lipophilic drugs during passage across the enterocyte. These aspects are discussed below.

After absorption into the enterocyte, lipid digestion products either diffuse directly across the cell and enter the portal vein, which leads to access to the systemic circulation by the liver, or are trafficked intracellularly to the endoplasmic reticulum (ER) where they are re-synthesized to triglycerides[25]. Re-synthesized triglycerides subsequently constitute a core lipid component of intestinal lipoproteins. Intestinal lipoproteins are large colloidal particles that consist of a hydrophobic core (which contains primarily triglyceride and cholesterol ester) and a more hydrophilic surface (which contains primarily phospholipid, free cholesterol and apolipoproteins, including apolipoprotein A-IV (REF. 21), B-48 (REFS 47,48), C-II, C-III (REFS 49–51) and E (REFS 52,53)). The main lipoproteins secreted by the enterocyte are chylomicrons and very low-density lipoproteins (VLDL). Lipoproteins assembled in the ER and Golgi subsequently fuse with the basolateral cell membrane of the enterocyte before release into the interstitial space. Following exocytosis from the enterocyte, the impermeability of the vascular endothelium to large colloidal particles combined with the large inter-endothelial gaps present in the lymphatic endothelium preferentially direct lymph lipoproteins towards selective uptake by the intestinal lymphatic system rather than the blood capillaries.

Similarly, drugs can be transported to the systemic circulation by either the portal vein or the intestinal lymphatic system following oral delivery (FIG. 4). Most low molecular mass drugs are absorbed through the portal vein as the rate of fluid flow in portal blood is approximately 500-fold higher than that of intestinal lymph. However, the lymphatic system can be a significant absorption pathway for highly lipophilic drugs. Although the exact mechanism(s) by which lipophilic drugs access the intestinal lymphatics is not completely understood, it is thought to involve drug association with lipoproteins

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Chylomicrons
Chylomicrons are larger (50–500 nM) and less dense (S_f > 400) lipoproteins than very low-density lipoproteins, and are formed exclusively in the small intestine following the ingestion of lipids (dietary-derived or formulation-derived).

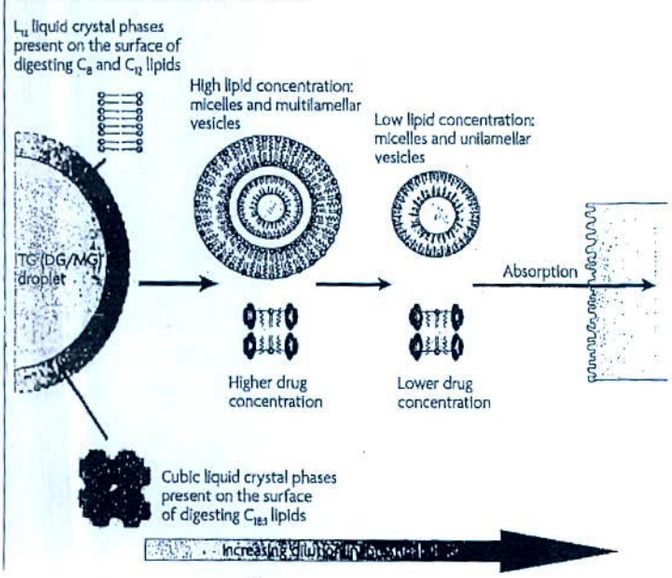
during transport through the enterocyte as lymphatically transported compounds such as DDT (dichlorodiphenyltrichloroethane)[54], aryl and alkyl hydrocarbons[55] and halofantrine[56] are transported in lymph within the apolar lipid core of lymph lipoproteins.

Charman *et al.* previously suggested that significant access to the intestinal lymphatic system will only occur for lipophilic drugs with adequate metabolic stability, with $\log D_{7.4}$ values greater than 5 and with solubilities in excess of 50 mg per g in long-chain triglyceride lipid[57]. Most drugs that are absorbed by the intestinal lymphatic system also demonstrate enhanced bioavailability when administered post-prandially. The nominal requirement for a high log D arises from consideration of the difference between portal vein blood and mesenteric lymph flow (500:1 volume per volume) and the realization that typically only 1% of lymph fluid is made up of lipid. Simplistically, the lipid flow to blood flow ratio is therefore of the order of 50,000:1 and drugs might be expected to require at least a 50,000-fold higher affinity for lymph lipid rather than blood to support substantive lymphatic transport (that is, log D > 4.7). The requirement for high drug solubility in long-chain triglyceride (> 50 mg per g) reflects the expectation that the majority of lymphatically transported drugs are solubilized within the apolar lipid core of lipoproteins.

Consistent with these guiding criteria, various highly lipophilic drugs and xenobiotics have been shown to be transported to varying degrees by the intestinal lymph following oral administration; including moxidectin[58], halofantrine[43,59,60], mepitiostane[61–63], testosterone derivatives[64], MK-386 (a 5α-reductase inhibitor)[65], penclomedine[42], naftifine[66], probucol[67], cyclosporine[68], ontazolast[69], CI-976 (REF. 70), fat-soluble vitamins and their derivatives, retinoids[71], lycopene[72], DDT and analogues[54,57], benzopyrene, and PCBs (polychlorinated biphenyls)[57].

Box 2 | Phase changes during the digestion of lipid-based formulations

It has been recognized for some time that digesting (food-derived) lipids display complex phase behaviour in aqueous fluids[212,227,228]. More recently, pseudo-tertiary phase diagrams have also been used to characterize and map the potential post-digestion phase behaviour of selected formulation lipids[30]. Examination of the changes in phase behaviour that occur as a function of lipid dilution in a model intestinal fluid provide information as to the phase changes that might be expected to occur as the products of digestion of formulation-derived lipids — such as, diglyceride (DG), monoglyceride (MG) and fatty acid — are formed on the surface of a lipid droplet and are diluted by, or incorporate, small quantities of intestinal fluid (see diagram). At a fatty acid: monoglyceride ratio of 2:1 (the expected stoichiometric ratio produced from the digestion of triglyceride (TG)), liquid crystalline phases dominate under conditions of low dilution and might, therefore, be expected to be present on the surface of a digesting lipid droplet. A lamellar (L_α) phase is evident in the presence of medium-chain lipid digestion products (C_8 and C_{12}), whereas a more viscous cubic (C) phase has been identified (in coexistence with a colloidal liquid (L1) phase) in long-chain lipid-containing systems ($C_{18:1}$). Further dilution of these liquid crystalline phases results in a phase change to an L1 system, which comprises large (and probably multilamellar) vesicular colloidal species. On further dilution the properties of the L1 phase increasingly mirror the colloidal composition of pure, simulated intestinal fluid (that is, coexisting mixed micelles and unilamellar vesicles). However, the vesicular species persist at lower lipid concentrations in the long-chain lipid system and more effectively retain drug solubilization capacity when compared with the medium-chain systems. Importantly, this highly dilute L1 phase is most likely to reflect closely the environment immediately adjacent to the absorptive surface of the enterocyte. Differences in phase-transition behaviour (that is, L_α to L1 or C to L1), the solubilization capacity of the phases formed and the potential effect of amphiphilic formulation components, including surfactant and co-solvents, on the nature of these species will probably dictate the patterns of solubilization (and eventual absorption) of poorly water-soluble drugs following digestion of lipidic formulations *in vivo*.



Implications of lymphatic drug transport

The unique anatomy and physiology of the intestinal lymphatic system provides a number of drug transport advantages when compared with portal blood transport. First, drugs that enter the mesenteric lymph are directly transported to the systemic circulation without first passing through the liver. As such, augmentation of drug uptake into the lymph system reduces the opportunity for hepatic first-pass metabolism and might therefore be a useful mechanism to enhance the bioavailability of drugs (for example, testosterone[64] (BOX 5)) in which significant hepatic first-pass metabolism is a limitation to (or in some cases precludes) oral bioavailability[59,64]. A recent study has also suggested that partition into the developing lipoprotein assembly pathways within the enterocyte might result in a reduction in enterocyte-based metabolism[73].

Drug access to the intestinal lymphatics might also alter systemic distribution patterns[43,70] and potentially toxicity and efficacy profiles. For example, B and T lymphocytes are transported around the body primarily by the lymphatic system and drugs that enhance or inhibit the immune system might therefore be more effective when absorbed by the intestinal lymph. The lymphatic system also has an important role in the dissemination of tumour metastases and acts as a reservoir for the human immunodeficiency virus (HIV)[74] — with recent evidence suggesting that HIV replication occurs largely in gut-associated lymphoid tissue[75]. It is possible therefore that increasing the concentration of anti-HIV and anticancer compounds in the mesenteric lymph might improve therapy.

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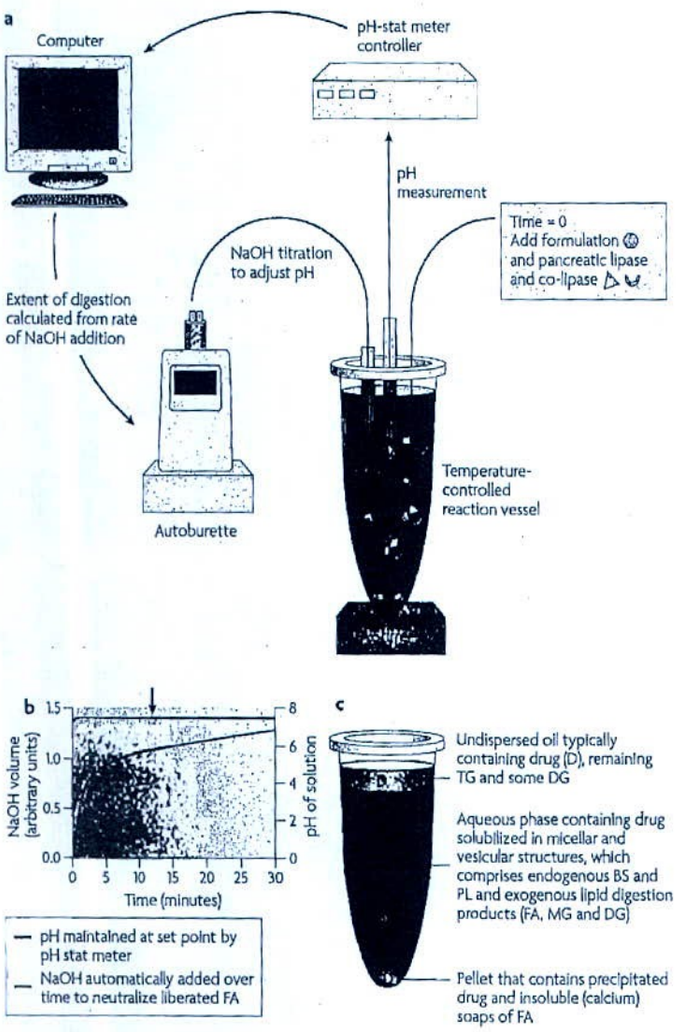


Figure 3 | **Lipid digestion models for in vitro assessment of lipidic formulations.** Lipid digestion models are being increasingly used as tools to facilitate improved *in vitro* evaluation of lipid-based drug delivery systems. Although the experimental details of these models differ slightly between laboratories, the basic principles of operation are similar. **a** | The models are built around a temperature-controlled (37°C) vessel that contains digestion buffer, bile salt (BS) and phospholipid (PL) (to represent a model intestinal fluid) into which lipid-based formulations are introduced, and digestion is initiated by the addition of pancreatic lipase and co-lipase. The onset of lipid digestion results in the liberation of fatty acid (FA), which in turn causes a transient drop in pH. **b** | The drop in pH is quantified by a pH electrode that is coupled to a pH-stat meter controller and autoburette, which together automatically titrate the liberated FA by the addition of an equimolar quantity of NaOH. This maintains the pH at a set point (thereby allowing the pH-sensitive process of digestion to continue) and facilitates indirect quantification of the extent of digestion (by quantification of the rate of NaOH addition and assumption of a stoichiometric reaction between FA and NaOH). **c** | Throughout the digestion process, samples can be taken and ultracentrifuged to separate the digest into a poorly dispersed oil phase, a highly dispersed aqueous phase and a precipitated pellet phase. Quantification of the mass of drug that is subsequently trafficked through to the highly dispersed aqueous phase and which does not precipitate provides an indication of the proclivity of the formulation with respect to *in vivo* precipitation and therefore a mechanism to (at least) rank-order the likely *in vivo* performance of a series of lipidic formulations. DG, diglyceride; MG, monoglyceride; TG, triglyceride.

Drugs in which a significant proportion of the dose is transported by the lymphatic system typically exhibit lymphatic drug concentrations two to three orders of magnitude higher than the corresponding plasma concentration, a situation that reflects a combination of limited drug distribution out of lymph, and a propensity for highly lipophilic lymphatic transport candidates to have large systemic volumes of distribution. Although the attainment of high lymphatic drug concentrations might provide therapeutic benefits, it is also apparent that elevated lymphatic drug concentrations and altered systemic disposition can also raise a number of safety implications. So, increased drug concentrations in the lymphatics have the potential to drive local (lymphatic) toxicity, and the delivery of high concentrations of lipoprotein-associated drug to the systemic circulation by the jugular vein (the main point of entry of lymph into the systemic circulation) could result in altered patterns of systemic exposure when compared to the absorption of non-lipoprotein-associated drug by the portal blood. This is important if formulation changes that occur during the course of a drug development programme (such as changes between lipid-free and lipidic formulations) might be expected to lead to significant changes to the route of delivery (portal versus lymphatic) to the systemic circulation. This is further complicated by the fact that the clinical development team might be essentially blind to the fact that changes to drug disposition and drug transport route have occurred, as overall systemic (plasma or serum) exposure is commonly the only quantifiable pharmacokinetic endpoint during clinical trial.

Models of intestinal lymphatic drug transport

Direct evaluation of intestinal lymphatic transport in humans is not practical owing to the non-reversible nature of the invasive surgery required to access the lymph, and the frailty of the lymphatic duct itself. Consequently, various animal models have been developed to estimate the likely intestinal lymphatic drug transport in humans[76–80]. These models rely on the collection of the lymph flowing from the intestine by the insertion of a cannula into either the mesenteric or thoracic lymph duct. The most common model for lymphatic transport studies is the rat and there are a wealth of historical transport data available. However, there are a number of methodological variations associated with the published data, including differences in the site of cannulation (mesenteric or thoracic lymph duct), animal treatment before and after surgery (fasting, pre-feeding lipid and length of recovery period) and the conscious state of the animal (conscious or anaesthetized), which complicate the direct comparison of data that arise from different models and experimental conditions[76]. More recently, a thoracic lymph-duct cannulated dog model has also been described[59,79], which offers an alternative method for estimating the likely lymphatic transport of lipophilic drugs in humans. In particular, it allows oral administration of clinically relevant full-sized human dose forms in representative fed and fasted states. The GI tract, gastric transit profile and biliary secretion patterns of the dog also reflect the human situation better when compared with the rat (in which bile is continuously secreted into the

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intestine). The pig might also provide an alternative to the dog as a suitable larger animal model[76,214]. Recently, a less invasive *in vivo* approach to the estimation of intestinal lymphatic drug transport has been described in which the systemic exposure of the drug is assessed after administration in the presence and absence of Pluronic-L81 or colchicine[81]. Pluronic-L81 and colchicine block intestinal chylomicron flow into the lymph and therefore inhibit lymphatic drug transport. This approach has the advantage of not requiring the surgical interventions inherent in lymph-duct cannulation; however, the broader implications of blocking chylomicron flow and intestinal lipid processing on overall drug exposure (and indirectly, lymphatic transport) have yet to be studied in detail.

An alternative approach to the use of animal models for the assessment of intestinal lymphatic transport is the use of cultured intestinal epithelial cells. In the drug development field, Caco-2 cells are well known as a high-throughput screen for the assessment of intestinal drug permeability, but this cell line is also used in lipid biochemistry to examine aspects of intracellular lipoprotein assembly[82]. Recently, Caco-2 cells have also been used to examine the influence of lipids and lipid-formulation excipients on drug incorporation into lipoproteins and the initial data are encouraging[83–85]. Although cultured cell models show considerable promise as a means for assessing the intracellular processing of lipids and drugs, and the potential impact of different excipients on this process, the predictive capacity of Caco-2 cells in estimating the extent of *in vivo* intestinal lymphatic drug transport has yet to be demonstrated.

Box 3 | Key formulation philosophies for lipids and lipophilic excipients

The choice of specific formulation components to provide optimal pharmaceutical and biopharmaceutical properties is drug specific and will depend on drug dose (potency) and the physicochemical properties of the compound concerned. A number of key principles, however, are apparent and should guide excipient selection.

Pharmaceutical properties

Solvent capacity. Molecularly dispersed formulations are typically preferred, whether dispersible, non-dispersible or destined for self-emulsification, and regardless of whether the dispersion is a solid (for example, a solid dispersion) or (more commonly) a liquid filled into hard or soft gelatin capsules. The solvent capacity of the formulation components is therefore a significant determinant of utility.

Impurity profiling. Lipidic formulations are often complex, in particular those that contain surfactants that are statistical mixtures of reaction products. Impurity profiling is therefore a crucial aspect of product quality. The presence of residual fatty acid in the formulation might be particularly important.

Solid-state properties. Lipid-based formulations can be solids, liquids or semi-solids. Although all can provide benefit in specific situations, interconversion from one physical form to another on storage leads to stability concerns in terms of both drug and excipient crystallinity and dissolution rate. Therefore, formulations for which components are 'all in' solution or 'all out' (that is, solid) simplify pharmaceutical aspects of product development.

In vivo solubilization properties

Maintenance of drug solubilization on dispersion. Lipid-based formulations should retain the drug in a solubilized state on initial dispersion in the gastrointestinal content and mixing with endogenous solubilizing species, including bile salts and phospholipids. This suggests that excipients with appreciable water solubility or water miscibility, in particular co-solvents, might constitute a risk to drug precipitation as their ability to maintain solubilization capacity on dilution is limited. However, the kinetics of drug precipitation are key and in some circumstances it might be possible to maintain the drug in a supersaturated state, after dilution of a co-solvent, for a period sufficient to support drug absorption.

Maintenance of drug solubilization on digestion. After dispersion, gastric and pancreatic lipase and co-lipase act at the surface of a lipid droplet to digest glycerides and potentially lipidic excipients, such as surfactants with susceptible (primarily fatty-acid ester) groups. The process of digestion can increase or decrease (which seems to be more common) the capacity of exogenous components to solubilize the drug, whether alone or in combination with endogenous bile salt or phospholipid. In this regard, the effect of digestion on drug solubilization is not readily predictable, although a growing body of evidence suggests that long-chain glycerides are less susceptible to loss of solubilization capacity on digestion, particularly for highly lipophilic drugs. Under these circumstances, *in vitro* models of lipid digestion provide useful tools for examining the generalized effects of digestion on formulation solubilization capacity (FIG. 3).

Biochemical properties

Potential for transporter and metabolism inhibition. Increasing evidence suggests the possibility of excipient (including lipid) effects on transporter function and metabolism within the enterocyte. In circumstances for which enterocyte-based metabolism or efflux provide a significant limitation to *in vivo* exposure, certain lipidic vehicles might therefore provide for increases in exposure beyond that expected by appreciation of solubilization benefits alone.

Stimulation of lymphatic transport. For highly lipophilic drugs, which exhibit an intrinsic capacity for intestinal lymphatic transport, judicious choice of formulation excipients (in particular the inclusion of a source of long-chain lipid) will enhance the proportion of the dose that is transported to the systemic circulation by the lymph, and could provide benefits in terms of reduced first-pass metabolism.

Enhancing lymphatic drug transport

Post-prandial state. Drug transport by the intestinal lymphatic system is significantly enhanced by co-administration with food. This reflects enhanced drug absorption into the enterocyte (owing to enhanced luminal solubilization) and the fact that a fatty meal substantially enhances intestinal lipoprotein assembly and therefore drug access to the intestinal lymphatic system. Changes to lymphatic drug transport following post-prandial administration can be dramatic. For example, the lymphatic transport of halofantrine increased from 1.3% to 53.9% of the administered dose when administered as a lipid-free solid dispersion to fed and fasted dogs, respectively[59]. In this study, the corresponding mass of lymphatic triglyceride recovered over 10 hours reflected the differences in lymphatic drug transport and was 32.6 g for fed dogs compared with just 0.5 g in fasted dogs. These types of studies in dogs (post-prandial studies are typically not possible in rodent models) therefore provide an indication of the maximal extent of lymphatic transport, and as such could be used as an initial indicator of the potential importance of lymphatic transport.

Lipid-based formulations. As lymphatic lipid and drug transport are closely related, lymphatic drug transport was historically believed to be marginal except when lymph lipid flux was increased by the administration of large quantities of lipid (such as those contained in a high fat meal). However, a recent study in fasted dogs has shown that administration of a single capsule of long-chain lipid can stimulate significant lymphatic transport of halofantrine (27% of the dose) after oral administration[86]. Interestingly, the mass of triglyceride recovered in the lymph over the 10 hours also suggested that administration of a small amount of exogenously

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Box 4 | The lymphatic system

The lymphatic system is a network of vessels (lymphatics), nodes and lymphoid tissues distributed throughout the body that typically shadows the architecture of the vascular system. Lymph is the fluid carried within the lymphatic system. The main physiological function of the lymphatic system is to maintain the body's fluid balance by returning excess fluid, proteins and waste products from the interstitial tissue to the blood circulation. Therefore, the lymphatic system is a one-way transport system from the interstitial tissue to the blood. The lymphatic system is also an essential component of the body's immunological defence system and interconnecting nodes and aggregates of lymphoid tissue within the lymphatic system disseminate and organize immune responsive cells. This Review describes specifically the intestinal (mesenteric) lymphatic system, which is responsible for the transport of absorbed dietary lipids (in the form of colloidal lipoproteins) and certain highly lipophilic compounds (for example, lipid-soluble vitamins and drugs) from the absorptive cells of the small intestine to the systemic circulation. Anatomically, the intestinal or mesenteric lymph drains into the cisterna chyli and is returned to the systemic circulation by the thoracic duct. Importantly, at least in terms of drug absorption and bioavailability, the mesenteric and thoracic lymph does not pass through the liver before entering the systemic circulation. This provides a route for drug transport that avoids the potential complications associated with the hepatic first-pass metabolism.

derived lipid (from the formulation) was able to support substantial lymphatic transport in the fasted state by recruiting endogenous lipid transport into the lymph.

Co-administration with lipids has been widely investigated as a means to enhance the lymphatic transport of highly lipophilic drugs. The basic premise underlying the use of these systems is that co-administration with lipids facilitates both the overall intestinal absorption of lipophilic drugs (by enhancing drug dissolution and solubilization in the intestinal milieu) and the extent of lymphatic drug transport (by stimulating lipoprotein formation and intestinal lymphatic lipid flux). The potential utility of lipids to enhance bioavailability and lymphatic transport can be assessed by considering lipid-chain length, lipid class, degree of saturation and degree of dispersion; these aspects have been reviewed previously[87,88].

In summary, fatty acids with chain lengths of 14 and above are primarily transported into intestinal lymph (although as much as 40% of a dose of long-chain fatty acid might be recovered in the portal blood in the form of triglyceride[89,90]) and shorter-chain fatty acids, which are more water soluble, more commonly diffuse across the enterocyte and are absorbed by the portal blood[91–93]. Predictably, therefore, the use of long-chain fatty acids has been shown to significantly increase drug transport into the lymph[43]. In general, monounsaturated and polyunsaturated fatty acids also promote lymphatic lipid transport more readily and produce larger lipoproteins when compared with saturated fatty acids[94–100].

In terms of lipid class (for example, fatty acid, monoglyceride, diglyceride, triglyceride or phospholipid), many studies have examined the effect of administering either fatty acid or triglyceride on the rate and extent of lymphatic drug transport[60,63,101,102]. In general, fatty acids are absorbed from the intestinal lumen without modification whereas triglyceride is first hydrolysed in the intestinal lumen before absorption. As such, there might be a time-lag associated with lymphatic transport from triglyceride vehicles. Other than glycerides, phospholipids and their digestion products have been shown to enhance the lymphatic uptake of halofantrine[103] and α-tocopherol[104] and could provide an avenue to enhanced lymphatic drug transport.

Lipid-based formulations with physical characteristics that are representative of the final stages of lipid digestion (for example, mixed micellar systems that contain lipid digestion products such as fatty acids and monoglycerides) rather than crude emulsions or lipid solutions have been shown to promote the lymphatic transport of lipophilic drugs such as halofantrine[105], CI-976 (REF. 70), mepitiostane[63], cyclosporine[106], *D*-α-tocopherol acetate[107], retinol, and retinyl palmitate[108]. The effect of the degree of dispersion of the formulation on lymphatic transport, however, is generally most evident after intraduodenal administration to anaesthetized animals in which gastric processing and intestinal mixing can be reduced[60,105]. In conscious animals the effect of formulation dispersion on drug absorption and lymphatic transport is less clear.

Lipidic prodrugs. Lipidic prodrugs comprise drugs that are covalently bound to a lipid moiety such as a fatty acid, diglyceride or phosphoglyceride, which is subsequently cleaved following uptake into the systemic circulation to liberate the parent drug[109]. In the case of glyceride or phospholipid-based prodrugs these systems are designed to mimic, and intercalate into, the glyceride or phospholipid re-synthetic pathways within the enterocyte. Lymph-directing prodrug strategies have been reviewed previously[109,110].

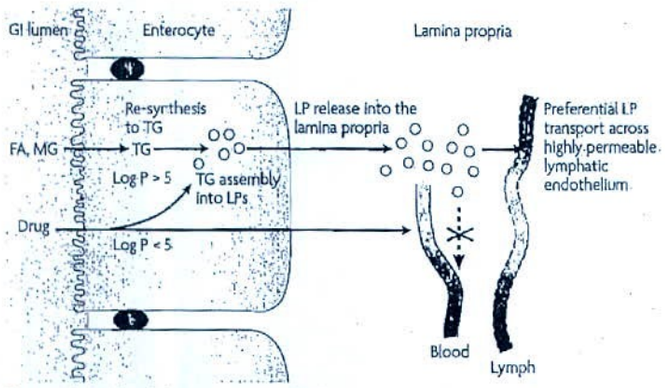


Figure 4 | **Lipid and drug transport by the mesenteric lymph or portal blood following oral delivery.** Following uptake into the enterocytes, lipid digestion products, for example fatty acid (FA) and monoglyceride (MG), can be re-synthesized to triglyceride (TG) in the smooth endoplasmic reticulum and subsequently assembled into TG-rich lipoproteins (LPs). LPs are exocytosed from the enterocyte into the lamina propria where the tight junctions between adjacent vascular endothelial cells and the presence of an underlying basement membrane preclude easy access to the blood capillaries. Instead, LPs preferentially access the lymphatics where adjacent endothelial cells overlap, which results in the formation of large inter-endothelial junctions with significantly enhanced permeability for colloidal species such as LPs. After absorption, drug molecules can also access the systemic circulation through uptake into either the portal vein or mesenteric lymphatic system. In most cases, drugs are transported to the systemic circulation by the portal vein as the rate of fluid flow in the portal vein is higher than that of the mesenteric lymph. However, for highly lipophilic drugs (typically with log P > 5 and solubility > 50 mg in long-chain TG lipid) partitioning into developing LPs in the enterocyte provides a preferential access mechanism to the intestinal lymph. Cellular junctions are depicted by green ovals. GI, gastrointestinal.

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Box 5 | Case study: testosterone

Orally administered testosterone is ineffective in the treatment of male androgen deficiency syndromes owing to almost complete pre-systemic metabolism in the enterocyte and liver. By contrast, testosterone undecanoate, the lipophilic undecanoate ester of testosterone, exhibits androgenic activity after oral administration in humans[61]. Administration of testosterone undecanoate, rather than testosterone, provides systemic exposure that is sufficient to provide androgenic activity because testosterone undecanoate is transported to the systemic circulation by the intestinal lymphatic system (rather than the portal vein) and thereby avoids first-pass hepatic metabolism. Testosterone (and the active metabolite 5α-dihydrotestosterone) are formed from testosterone undecanoate after systemic hydrolysis. Although lymphatic transport cannot be examined directly in humans, in dogs more than 80% of the systemically available testosterone that results from administration of testosterone undecanoate has been shown to result from systemic hydrolysis of lymphatically transported testosterone undecanoate[61]. Administration of the lipophilic ester prodrug of testosterone therefore facilitates the oral administration of testosterone, which would otherwise be prevented by the complete first-pass metabolism.

Lipids and changes to enterocyte biology

The potential for lipids and lipidic excipients to interact with lipid and drug transport pathways into and across the enterocyte has emerged as a growing area of interest[61,85,103,111–113]. In particular, lipids and lipidic excipients seem to interact with and potentially inhibit the activity of lipid (and drug) transporters present on the apical (and possibly basolateral) membrane, regulate the expression of lipid-binding (and drug-binding) proteins within the cytosol, and effect considerable change to the intracellular pooling of lipids within the enterocytes.

Apical membrane lipid transporters. The products of lipid digestion are absorbed across the apical membrane into the enterocyte by active[114,115] and passive transport[115] (FIG. 5). Uptake is believed to be largely carrier-dependent at low lipid concentrations and primarily passive at higher fatty-acid concentrations[115]. A number of potential apical membrane fatty-acid transporters have been identified, including CD36 and the fatty-acid transporter (FAT)[117–119], plasma membrane fatty-acid-binding protein (FABPpm)[114,120], fatty-acid transport protein 4 (FATP4)[121], scavenger receptor BI[122], GP330 (also known as low-density lipoprotein-related protein 2)[123] and caveolin[124]. Similarly, apical membrane transporters for monoglyceride have been described[125–127]. A number of transport proteins have also been implicated in cholesterol uptake across the enterocyte plasma membrane, including scavenger receptor BI[122,128], caveolin[124], CD36 and FAT[117], and NPC1L1 (Niemann–Pick disease, type C1, gene-like 1)[129,130]. The quantitative importance of these transporters in the overall process of intestinal lipid absorption has yet to be fully elucidated.

Few studies have addressed the role of these endogenous lipid transporters in the passage of lipophilic drugs across the apical membrane. In light of the more clearly demonstrated role of apical membrane transporters for amino acids and oligopeptides[131], monosaccharides[132], organic cations[133], organic anions[134], and bile acids[135] in the transport of drugs, which resemble their endogenous ligands[136,137], it is possible that lipid transporters might provide a similar functionality. However, it is also important to remain cognizant of the inherently high passive permeability of the majority of the highly lipophilic drugs that are likely candidates for formulation in lipidic dose forms.

Apical membrane efflux transporters. In addition to facilitating passage of compounds across the apical membrane into the enterocyte, an increasing number of transport proteins have been identified that efflux material back into the intestinal lumen following absorption. These transporters most commonly belong to the ATP-binding cassette (ABC) superfamily of proteins, which, as a wider group, comprise membrane transporters, ion channels and receptors[138]. Transporters that have been more frequently implicated in the intestinal efflux of drugs and lipid molecules include the multidrug resistance (MDR) transporter family, multidrug resistance-associated protein (MRP) transporter family and breast cancer resistance protein (BCRP from the *ABCG2* gene)[139,140].

Perhaps the most widely studied is P-glycoprotein (P-gp; also called MDR1 or ABCB1), which was the first of the ABC transporters to be identified. P-gp is well established as a mediator of drug efflux across the apical membrane of enterocytes and can therefore act as a barrier to the oral absorption of P-gp substrates. P-gp is able to bind and transport a broad range of drug substrates but shows some preference for lipophilic cations. P-gp and other ABC transporters have also been implicated in plasma membrane lipid transport and/or intestinal lipoprotein formation[83,141,142]. This revelation follows the discovery that MDR2 is the primary transporter of phospholipid into bile[143] and that cholesterol exsorption from enterocytes occurs in association with ABCA1 (REF. 144), ABCG5 and ABCG8 (REF. 145). It has been postulated that P-gp, in addition to potentially reducing drug absorption through apical membrane efflux, might also enhance intestinal lymphatic drug transport by influencing intestinal lipoprotein formation and secretion[83]. However, the exact role of P-gp and the ABC transporters in lymphatic lipid and drug transport remains to be confirmed.

Cytosolic lipid-binding proteins. After absorption across the apical membrane, lipid digestion products can cross the enterocyte by diffusion around the apical and basolateral membranes or can diffuse across the cytosol either alone or through association with intracellular lipid-binding proteins (iLBPs)[146]. Cytosolic lipid-binding proteins in the small intestine include intestine and liver fatty-acid-binding protein (L-FABP)[144,146–148], sterol carrier proteins (SCP)[149,150], retinol and retinoic-acid-binding proteins[151], and ileal bile-acid-binding protein (I-BABP)[148]. It has been suggested that lipid-binding proteins might facilitate the diffusion of lipids across enterocytes[152] and/or act as cytosolic storage 'sinks' for excess, and potentially toxic, quantities of free fatty-acid and exogenous substances[153]. However, it is still unclear which of these processes predominates *in vivo* and whether different binding proteins perform either or both of these functions. In terms of the capacity of binding proteins to influence lipophilic drug absorption, we have recently shown that some lipophilic drugs

VLDL
Very low-density lipoproteins (VLDL) are small (20–50 nM) and more dense (S_f = 20–400) lipoproteins than chylomicrons, and are formed in the liver and the small intestine (where they are the predominant lipoproteins secreted in the fasted state).

Log $D_{7.4}$
Log_{10} of the octanol–water partition co-efficient of a molecule (for example, a drug) at pH 7.4.

Pluronic L-81
A hydrophobic surfactant that blocks intestinal chylomicron secretion at the pre-Golgi level without affecting triacylglycerol uptake into the enterocytes, or triglyceride re-esterification in the smooth endoplasmic reticulum.

Colchicine
Clinically used to treat gout and has also been shown to cause accumulation of lipoproteins in the smooth endoplasmic reticulum and Golgi, thereby blocking chylomicron exit from the enterocytes.

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Box 6 | Nuclear hormone receptors and lipid transport protein expression

The nuclear hormone receptors are a family of regulatory proteins, which include the classic endocrine receptors that mediate the actions of steroid hormones, thyroid hormones and fat-soluble vitamins (A and D)[229]. They also include the more recently discovered orphan nuclear receptors[230,231], some of which function as lipid sensors that regulate the expression of proteins involved in lipid metabolism and transport[184]. These orphan nuclear receptors (and some known ligands) include: the peroxisome proliferator-activated receptor (PPAR) for fatty acids[232]; liver X receptor (LXR) for oxysterols[233,234]; farnesoid X receptor (FXR) for bile acids[234]; steroid and xenobiotic receptor (SXR) and pregnane xenobiotic receptor (PXR) for steroids and xenobiotics[235]; and constitutive androstane receptor (CAR) for xenobiotics[236]. PPARα, PPARδ and PPARγ are of particular interest in intestinal lipid and drug absorption as they control the expression of a number of proteins, including fatty-acid-binding proteins (FABPs), which are involved in lipid and potentially drug transport and metabolism[232]. Specifically, PPARα is believed to function as a global regulator of fatty-acid metabolism and catabolism. Ligands for PPARα include indomethacin, LTD4 antagonists (for example, LY171993), and fibrate drugs (GW2331, clofibric acid and fenofibrate). Natural ligands for PPARα include fatty acid (both medium-chain and long-chain unsaturated) and eicosanoids and their metabolites. PPARγ has a role in adipogenesis, cell differentiation, insulin sensitization, atherosclerosis and cancer, and as such the antidiabetic thiazolidinedione drugs — for example, rosiglitazone (Avandia; GlaxoSmithKline) — are strong ligands for this receptor[232]. The ligands for PPARγ also include indomethacin, LTD4 antagonists and fibrates (although the fibrates are weaker ligands for PPARγ than for PPARα), and natural ligands for PPARγ include arachidonic acid metabolites, fatty acid and triterpenoids. PPARδ has a less well understood function[237–239] and ligands include essential long-chain fatty acids, iloprost, carbaprostacyclin and some fibrates. PPARδ agonists seem to induce liver fatty-acid-binding protein (L-FABP) in the small intestine whereas PPARα seems to regulate L-FABP expression in the liver[182]. It is interesting to speculate that as these ligands — which activate these regulatory proteins — influence the expression of genes involved in lymphatic lipid transport, they could also affect the efficiency of lymphatic drug transport.

bind to intestinal fatty-acid-binding protein *in vitro*[112], and that drugs with molecular structures most similar to the endogenous ligand (fatty acid) seem to bind with highest affinity. The affinity of lipophilic drugs for other lipid-binding proteins and the relevance of these processes to drug passage across the enterocyte cytosol, and ultimately oral lipophilic drug absorption, are currently under examination. At the very least, however, cytosolic lipid-binding proteins are likely to alter the intracellular disposition of lipophilic drugs indirectly through their effect on intestinal lipid absorption and pooling within the enterocyte[154].

Lipids and drug transport proteins

The potential for lipidic excipients to attenuate the activity of efflux proteins such as P-gp has generated considerable recent interest[155,156]. Although several ethoxylated lipids and surfactants have been shown to inhibit drug efflux by P-gp *in vitro*[157–169], few studies have managed to conclusively assign an enhancement of drug absorption *in vivo* to a formulation-related effect on the functionality of P-gp[170]. One exception is a recent study that described an inhibitory effect of tocopheryl polyethylene glycol 1,000 succinate (TPGS) on the efflux of talinolol by P-gp *in vitro* and subsequently showed an enhancement in the oral bioavailability of talinolol in humans[171]. The potentially multi-faceted effect of surfactants on drug absorption, however, adds complexity to the assessment of the likely effects of solubilizing excipients on membrane transport processes. So, delineation of the contributions to enhanced drug absorption that result from changes to drug solubilization in the intestinal milieu and modulation of efflux transporter activity *in vivo* are complex. Also, even *in vitro* the inevitable reduction in thermodynamic activity that the inclusion of a solubilizing species in a transport buffer effects requires careful data interpretation[166,172]. In terms of lymphatic drug transport, the situation is complicated further as recent studies have suggested that certain lipophilic surfactants could inhibit P-gp and simultaneously increase drug absorption and reduce intestinal lipoprotein secretion. Under these circumstances the net effect on lymphatic drug transport is difficult to predict[83,84].

In addition to the potential effect of lipidic excipients (primarily surfactants) on P-gp-mediated efflux, Benet and co-workers have recently suggested that high-fat meals could inhibit P-gp[173] and studies by Konishi *et al.* have shown increased accumulation of a P-gp drug substrate in Caco-2 cells on incubation of the cells with monoglyceride[174,175]. However, the potential for direct inhibition of P-gp by food-related components remains controversial and requires a more complete mechanistic explanation. Interestingly, high-fat meals also stimulate the release of biliary lipids and these studies are therefore consistent with others that suggest that intestinal fluids, which contain bile salt and phospholipid, inhibit P-gp-mediated drug efflux[176]. This raises the intriguing possibility that dietary or formulation lipids might be capable of affecting oral drug absorption by direct effects on drug efflux transporters, and also by an indirect effect on transporter activity mediated by biliary secretion.

The levels of expression of apical membrane lipid transporters[177,178], cytosolic lipid-binding proteins[148,179] and ABC efflux transporters[138] are dynamically regulated both by endogenous and by exogenous ligands, which include drugs, formulation and dietary components such as lipids. This may be regulated at both the transcriptional or post-transcriptional level. However, one pathway of particular interest is that of ligand interaction with one or more of the family of nuclear hormone receptors (NHRs). NHRs have generated considerable interest recently in the pharmaceutical sciences with the realization of their role in the regulation of detoxification systems, such as cytochrome P450 metabolic enzymes and P-gp[180]. However, NHRs may also be important in the context of lipid and lipophilic drug absorption, as they regulate, in response to lipids, the transcription of proteins involved in lipid trafficking and metabolism (BOX 6). In particular, FABPs in the small intestine are upregulated at the transcriptional level by chronic exposure to increased quantities of dietary lipids[117,147,179,181–183] through lipid interaction with an NHR complex[184]. By contrast, L-FABP levels are reduced following presentation of a chronic low-fat diet to rats[147]. The effects of acute changes to FABP expression have been studied in less detail[154,181,185]. However, even small, formulation-relevant lipid doses seem to be able to acutely (within hours) upregulate the expression of I-FABP and L-FABP in the small intestine of rats[154]. As FABPs have been shown to bind to non-endogenous ligands, and in normal usage few formulations are administered as a

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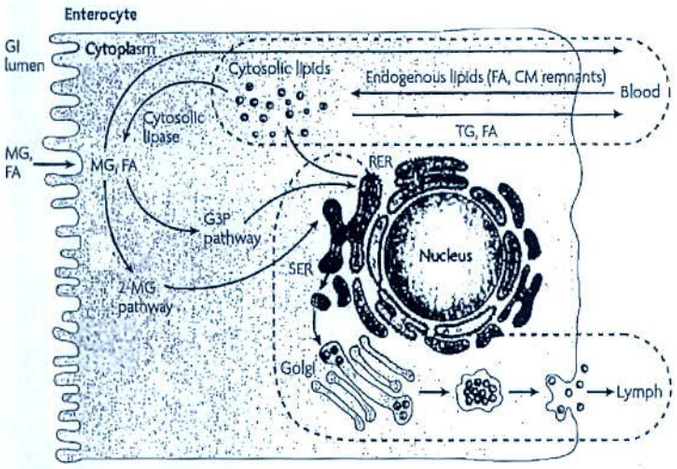


Figure 5 | **Pathways of lipid absorption and pooling within the enterocyte.** Following uptake across the apical membrane of the enterocyte, the products of gastrointestinal (GI) lumen lipid digestion — monoglyceride (MG) and fatty acid (FA) — can either diffuse across the enterocyte and enter the portal vein blood[89] or be re-synthesized to triglyceride (TG) by either the 2-monoglyceride (2-MG) pathway associated with the smooth endoplasmic reticulum (SER) or the α-glycerol-3 phosphate (G3P) pathway associated with the rough endoplasmic reticulum (RER)[193,195]. TG formed by these pathways typically enters the ER lumen and is assembled into lipoproteins (LPs; represented by orange circles). LPs are then transported to the Golgi, exocytosed from the enterocyte and taken up into the intestinal lymphatic system[207]. As lipid contained within the lipoprotein assembly pathways and the Golgi is destined for transport to the systemic circulation by the intestinal lymphatic system, this pool of lipids is referred to as the lymph lipid precursor pool (dashed blue line)[103,199]. A cytosolic pool of lipids is also located within the enterocyte[103,199]. This lipid pool comprises excess TG formed by the G3P pathway[198] and endogenous lipids taken up from the intestinal blood supply in the form of either FA or chylomicron (CM) remnants[186,188]. The cytosolic lipids are subject to hydrolysis by cytosolic lipase[200] and the digestion products formed can be re-circulated into TG assembly pathways[195]. However, the majority of lipids from this pool exit the enterocyte in the form of TG or free FA and are taken up into portal vein blood[103,199]. The pool of lipids that is transported from the enterocyte by the portal vein is therefore referred to as the portal lipid precursor pool (dashed red line)[103,199]. Recent evidence suggests that the trafficking and pooling of lipids within the enterocyte have a significant influence on the intracellular disposition of lipophilic drugs[103].

single dose therapy, this study raises the possibility that dietary lipids and lipid-based formulations might alter lipophilic drug absorption and intracellular disposition through alterations to expression levels of lipid-binding and transport proteins within the enterocyte.

Lipid trafficking in the enterocyte

FIGURE 5 depicts the known pathways of lipid absorption and pooling within the enterocyte. Orally administered exogenous lipids enter the enterocyte through the apical membrane, whereas endogenous lipids can access the enterocyte through either the apical or basolateral membranes[186,187]. Apically sourced endogenous lipids include those in bile and lipids derived from desquamated enterocytes. Basolaterally sourced endogenous lipids include fatty acid and chylomicron remnants taken up from the intestinal blood supply[186,188]. Endogenous lipids can also be synthesized *de novo* in the enterocytes[187]. Of these potential sources of endogenous lipid, apically sourced, biliary-derived lipids are the main contributor to lymphatic lipid transport (~50% in the fasted state in rats) and bile diversion substantially reduces fasted and post-prandial lymphatic lipid transport[189–191].

Following uptake into the enterocyte, apically sourced exogenous and endogenous lipids either diffuse across the cytosol and enter the portal vein, or migrate to the ER where re-synthesis to tryglyceride occurs. Exogenous monoglyceride and fatty acid are primarily re-synthesized to triglyceride by the monoglyceride pathway, which consists of a group of enzymes (triglyceride synthetase) present on the surface of the smooth ER (SER)[192–195]. A second pathway for triglyceride synthesis, the α-glycerol-3-phosphate (G3P) pathway[193,195] is believed to be located on the rough ER (RER) rather than the SER[195]. The contribution of the monoglyceride and G3P pathways to triglyceride synthesis depends on the supply of monoglyceride and fatty acid. During digestion and absorption of a significant quantity of exogenous triglyceride (for example, post-prandially), in which monoglyceride is present in high concentrations, the G3P pathway is inhibited and monoglyceride is primarily (80%) converted to triglyceride by the monoglyceride pathway[193,195]. Triglyceride synthesized by the monoglyceride pathway crosses the SER membrane[196,197] and is subsequently assembled into lipoproteins (see BOX 7 for details). By contrast, in the absence of a reasonable quantity of monoglyceride (such as in the fasted state or following fatty-acid administration alone) the G3P pathway located on the RER membrane is the main pathway of triglyceride synthesis[25,195]. A small proportion of the triglyceride produced by the G3P pathway is incorporated into the lipoprotein assembly process in the RER and provides a source of lymphatic triglyceride in the fasted state. However, the majority enters a cytoplasmic pool of triglyceride droplets[25,195,198].

Basolaterally sourced endogenous lipids taken up from the intestinal blood supply also enter the cytoplasmic lipid pool. These cytoplasmic lipid droplets (which are not protected by a surrounding membrane) are diffusely distributed across the enterocyte cytoplasm and are continuously hydrolysed to monoglyceride and fatty acid[195,198,199] by cytosolic lipase[200]. Once hydrolysed, some of these lipids can be re-synthesized to triglyceride by the monoglyceride pathway and incorporated into lipoprotein assembly pathways in the SER[195,199]. The majority of the lipids in the cytoplasmic lipid pool, however, are transported from the enterocyte to the systemic circulation by the portal vein as either free fatty acid or triglyceride and the pool has therefore been referred to as the portal lipid precursor pool[103] or mucosal storage pool[199,201]. By contrast, lipids within the enterocyte, which enter lipoprotein assembly pathways through the ER and Golgi and are destined for transport by the lymph, can be referred to as the lymph lipid precursor pool or chylomicron precursor pool[201]. Owing to the close relationship between the biosynthetic pathways of the lipids in the portal and lymph lipid precursor pools, the size and dynamics of these pools are interrelated. For example, Nevin *et al.* have shown that the size of the portal lipid precursor pool is inversely proportional to the efficiency of lymphatic lipid transport[201].

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Box 7 | Models of VLDL and CM assembly

Two models have been suggested to explain very low-density lipoprotein (VLDL) and chylomicron (CM) assembly pathways in the enterocyte. In the first model for assembly of VLDL and CM (the independent model), it has been proposed that the formation of intestinal VLDL and CM occurs by two independent pathways[100,240]. Therefore, assembly of the smaller VLDL particles occurs constitutively, whereas CM assembly is induced in the post-prandial state. In the second or sequential assembly model[241,242] lipoproteins are believed to be synthesized by three discrete and independent steps: assembly of primordial lipoproteins by association of apolipoprotein B-48 with phospholipid and neutral lipids from the rough endoplasmic reticulum (RER) membrane, and subsequent transfer to the RER lumen in a process facilitated by microsomal triglyceride transport protein (MTP)[196,197]; synthesis of triglyceride-rich droplets in the smooth endoplasmic reticulum (SER) — the size of which increases in the post-prandial state; and 'core expansion' in which the primordial lipoprotein fuses with the triglyceride droplets at the junction of the SER and RER, which leads to the formation of a 'nascent lipoprotein'. After assembly in the endoplasmic reticulum, lipoproteins are transported to the Golgi apparatus, exocytosed from the enterocyte and taken up into the lymph. Further addition of exogenous lipid and glycosylation of apolipoproteins can occur in the Golgi[242].

Exogenous lipids and enterocyte lipid pools

In the fasted state, only a small quantity of lipid is found in the portal and lymph lipid precursor pools[201]. Ingestion of formulation or dietary lipid, however, alters the composition, size and turnover rate of both lipid pools[103,201,202], and, therefore, the intracellular disposition of highly lipophilic drugs that have intrinsically high affinity for intracellular lipid domains. Although research into the effect of altered lipid-pooling profiles on intracellular drug disposition is in its infancy, we have recently shown in lymph-cannulated rats that the mass, turnover rate and composition (endogenous or exogenous) of lipids in the lymph lipid precursor pool dramatically alters the uptake of a model lymphatically transported drug (halofantrine) into the lymph lipid precursor pool, and subsequently alters the rate and extent of lymphatic drug transport[103]. In particular, following administration of high lipid doses, exogenous fatty acids were the primary drivers of lymphatic drug transport, whereas following administration of lower lipid doses, the lymph lipid precursor pool and lymph contained primarily endogenous fatty acid. Consistent with a previous study, recruitment of biliary-derived endogenous fatty acid into the lymph led to an enhancement of lymphatic drug transport, whereas recruitment of an alternative source of endogenous fatty acids into the lymph (presumably basolaterally sourced fatty acid from the intestinal blood supply) did not support lymphatic drug transport[113]. Interestingly, in both studies lysophosphatidylcholine was able to expand the lymph lipid precursor pool and significantly enhance lymphatic drug transport when administered with high lipid dose formulations. Lysophosphatidylcholine might therefore be a useful excipient to facilitate enhanced lymphatic uptake of lipophilic drugs.

Changes to intracellular drug disposition, and in particular access to the lymph lipid precursor pool, that occur as a result of administration of exogenous (formulation- or food-related) lipid are therefore expected to profoundly influence the extent of lymphatic drug transport. A recent study has also suggested that changes to the nature of intracellular lipid pools and intracellular drug partitioning might alter the susceptibility of a drug to an enterocyte-based metabolism[73]. Clearly, the intracellular trafficking and pooling of lipids are complex and highly regulated processes. Although early studies suggest that small, formulation-sized lipid doses might alter the intracellular pooling of lipids, and therefore affect intracellular drug disposition, further research is required to define how important these changes are in the context of the overall absorption, bioavailability and lymph–portal partitioning of lipophilic drugs.

Summary and perspectives

In the future, highly lipophilic, poorly water-soluble drug candidates will remain common outcomes of drug discovery programmes in spite of the vigorous application of contemporary lead optimization programmes. Although such candidates will probably have excellent *in vitro* potency and biological selectivity, exposure after oral administration is expected to remain limited by poor dissolution and solubilization. Lipid-based drug delivery systems offer one approach to enhance the absorption of this class of drug candidate. However, an improved understanding of the formulation and biopharmaceutical complexities of these molecules is required to drive the rapid completion of successful development programmes.

Central to the potential utility of lipid-based formulations as a means to enhance the bioavailability of a drug candidate is the lipophilicity of the drug candidate itself. In this regard, traditional lead optimization programmes are typically directed towards the design of biologically active and selective candidates with sufficient lipophilicity to provide adequate membrane permeability, absorption and distribution, while retaining reasonable aqueous solubility. In many instances, however, the binding energetics of putative biological targets and receptors drives candidate design towards an iterative endpoint of relatively hydrophobic drug candidates in which aqueous solubility characteristics are optimized but remain poor. The recognition that lipid-based dose formulations can provide acceptable oral bioavailability for even extremely poorly water-soluble drugs is encouraging, but needs to be tempered by the realization that many such compounds have low solubility in lipids, thereby limiting the dose that could be dissolved in a unit formulation. Therefore, during lead optimization it might be useful to explore more lipophilic (as opposed to simply hydrophobic) candidates as this could confer greater intrinsic potency and provide higher lipid solubility (and therefore enable the use of lipid-based formulations to provide enhanced exposure). This strategy further enhances the capacity to explore the structure–activity relationships of relevant scaffolds and could provide unique avenues of protection for the intellectual property base surrounding the drug discovery programme. Conversely, potentially negative aspects of more lipophilic drug candidates include the potential for increased drug metabolism (although this

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can be readily screened *in vitro*), the reliance on 'atypical' lipid-based formulations during preclinical and clinical development with the allied possibility that oral bioavailability becomes highly formulation-dependent, and an overall increase in the complexity (perceived or otherwise) of the drug development programme. Nonetheless, in situations in which exploration of hydrophilic functional groups on a lead scaffold leads to crucial losses in potency or selectivity, an understanding of the potential utility of lipidic formulations to enhance and regulate the absorption of highly lipophilic drugs (in conjunction with an awareness of the effect of formulation components on transporter functionality, intracellular drug disposition and lymphatic transport) may offer a means of addressing these otherwise intractable developmental issues.

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Competing interests statement
The authors declare no competing financial interests.

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