

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 13-236457- -00008-0000

Fecha: 2015-01-30 17:08:17 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 523 RESPUESTA Folios: 53

Señor Director
DIRECCIÓN DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

MF

Trámite : 011
Evento : 378
Actuación : 523

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. Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, me refiero al oficio No. **13642** notificado por fijación en lista el 4 de Noviembre de 2014.

2. Mediante oficio No. **13642** se puso en conocimiento del solicitante el informe que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente. Las observaciones realizadas en el oficio se refieren a la claridad del pliego reivindicatorio, a la oposición presentada, al nivel inventivo de las reivindicaciones 1 a 19 en vista de los documentos **D1**: WO9906024 y **D2**: US20080306100; y al nivel inventivo de las reivindicaciones 1 a 18 en vista de los documentos **D1** y **D3**: Biomedicine & Pharmacotherapy 58 (2004) 173 - 182.

3. **Respuesta a las objeciones**

De este modo y con el objeto de atender las objeciones arriba mencionadas, se desea presentar las siguientes modificaciones, aclaraciones y argumentos:

"EXCELENCIA LEGAL EN UN MUNDO SIN FRONTERAS"

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3.1. Con respecto a la oposición presentada por Lafrancol

En primer lugar, deseo aclarar que ni a mí ni a mi representada nos fue notificada oposición alguna presentada durante el trámite de la solicitud. De esta forma, mi representada no tuvo oportunidad de responder oportunamente a la oposición, lo que claramente es una violación al derecho al debido proceso y va en contra del artículo 43 de la Decisión 486 de la Comunidad Andina de Naciones.

En vista de lo anterior, ninguno de los argumentos o documentos citados en la oposición presentada por Lafrancol puede ser utilizado para desvirtuar la patentabilidad de la presente solicitud. En este orden de ideas, el documento US2008306100, identificado en el oficio como D2, no debería ser usado por el examinador para afectar los requisitos de patentabilidad de la presente solicitud.

Sin perjuicio de lo anterior y sin ánimo de reconocer la validez de las objeciones comunicadas en el oficio **13642**, a continuación se presentan argumentos a favor de la patentabilidad de la presente solicitud considerando D2 como estado de la técnica. Esto únicamente con el fin de agilizar el trámite de la solicitud.

3.2. Modificación de una solicitud en trámite

De acuerdo con el artículo 34 de la Decisión 486 de la Comisión de la Comunidad Andina, donde se establece que la modificación de una solicitud es posible en cualquier momento del trámite mientras no implique una ampliación de la solicitud inicial, me permito presentar un nuevo pliego reivindicatorio conformado por 26 reivindicaciones.

El nuevo pliego reivindicatorio corresponde esencialmente al anterior, pero se hicieron las siguientes modificaciones:

- Se incluyó la materia de las reivindicaciones 2, 4 y 12 en las reivindicaciones independientes 1 y 25.
- Se incluyó la materia de la reivindicación 10 en la reivindicación 8.
- Se eliminó la reivindicación 11.

- Cada una de las modalidades preferentes ha sido definida en una reivindicación adicional.

3.3. Argumentos a favor de la claridad del pliego reivindicatorio

En primer lugar, y como se mencionó anteriormente, las nuevas reivindicaciones 1 y 26 incluyen ahora las limitaciones de las reivindicaciones 2, 4 y 12, con algunos rangos o posibilidades restringidos a las realizaciones preferentes y no a los rangos más amplios del pliego reivindicatorio anterior. También se ha delimitado el alcance de las reivindicaciones 5 y 8 a un grupo más conciso de elementos.

Con respecto al soporte en la descripción, es preciso mencionar que la descripción divulga explícitamente, e incluye ejemplos específicos de, lo siguiente:

- Una pluralidad de fases lipofílicas tales como ácido oleico (ejemplos 1 a 34), ácido ricinoleico (ejemplos 35 a 37), triglicéridos de cadena media (ejemplos 36, 38, 39), aceite de linaza (ejemplo 37), monocaprilocaprato de glicerol (ejemplos 44 a 46), aceites de oliva, girasol y canola (ejemplo 49), y monooleato de glicerilo (ejemplos 50 a 52).
- Una pluralidad de surfactantes tales como polioxil-35-aceite de ricino (todos los ejemplos), polioxil 8 glicérido de caprilocaproilo (ejemplos 23 a 25), laurilsulfato de sodio (ejemplo 39), y monocaprilocaprato de glicerol (ejemplos 35, 36 y 38).
- Una pluralidad de co-surfactantes tales como caprilocaproil polioxil-8 glicéridos (ejemplo 4), glicéridos caprílicos (ejemplo 5), monooleato de sorbitán (ejemplo 6) y PEG-6-linoleato de glicerilo (ejemplo 7).

Es preciso mencionar que las reivindicaciones modificadas son una generalización razonable de los ejemplos, ya que todas las posibilidades cubiertas comprenden el efecto técnico sorprendente que se explicará más adelante en la defensa del nivel inventivo.

En adición a lo anterior, se ha incluido el nombre químico del compuesto NRC-AN-019 en el nuevo pliego reivindicatorio con el fin de favorecer la claridad de éste.

Finalmente, los términos “preferiblemente” y “muy preferiblemente” han sido eliminados del pliego reivindicatorio y se ha definido cada modalidad preferente en una nueva reivindicación dependiente.

En consecuencia de lo anterior, se considera que las objeciones formuladas en cuanto a la falta de claridad de las reivindicaciones han sido superadas.

3.4. Argumentos a favor del nivel inventivo de la presente solicitud

Los documentos D1 y D3 describen el principio general de formular fármacos hidrofóbicos con una fase lipofílica (como por ejemplo un aceite como los de D3 o una combinación de glicéridos como las de D1), un surfactante y un solvente con el fin de aumentar la biodisponibilidad. Por su parte, D2 divulga el compuesto NRC-AN-019.

Sin embargo, no existe ninguna motivación en el arte previo que inclinara a una persona versada en la materia a combinar D1 o D3 con las enseñanzas de D2. En particular, no hay razón alguna por la que un técnico versado en la materia que quisiera formular el compuesto NRC-AN-019 acudiría a los documentos D1 o D3. Lo anterior debido a que D1 y D3 mencionan explícitamente que el uso de una fase lipofílica, un surfactante y un solvente es únicamente aplicable a principios activos altamente lipofílicos con baja solubilidad en agua, mientras que el compuesto NRC-AN-019 no es lipofílico y no es soluble en agua.

Específicamente con respecto a D1, se debe mencionar que éste describe formulaciones auto-emulsificantes para un grupo de compuestos farmacéuticos de piranona. Dicho documento explica, en los renglones 22 a 27 de la página 1, que los compuestos de piranona son lipofílicos, con solubilidad limitada en agua, y por lo tanto tienen una muy baja biodisponibilidad. Las formulaciones de D1 atacan este problema mediante una mezcla de lípidos (particularmente ciertos mono y diglicéridos), un solvente y un surfactante.

D1 pone gran énfasis en que los compuestos de piranona sean lipofílicos. Por lo tanto, el fármaco formulado es descrito consistentemente como lipofílico. Las enseñanzas del artículo "Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs" de Christopher J.H. Porter *et al* (adjunto) refuerzan el requerimiento de D1 según la cual el agente activo en la formulación debe ser lipofílico. El abstract del artículo mencionado especifica que:

"es incrementalmente aparente que las formulaciones que contienen lípidos naturales y/o sintéticos presentan medios viables para mejorar la biodisponibilidad oral de algunas drogas con pobre solubilidad en agua, altamente lipofílicas"
(énfasis añadido)

También se debe aclarar que aunque los compuestos de piranona de D1 sean altamente lipofílicos, éstos son al menos parcialmente solubles en agua. Por lo tanto, Los compuestos de dicho documento evidencian cierta solubilidad en agua, como se menciona en el renglón 12 de la página 5 de D1, así sea baja.

Ahora con respecto a D3, este documento se refiere al uso de sistemas de administración de fármacos auto-emulsificantes (SEDDs) para formular compuestos farmacéuticos altamente lipofílicos (abstract, renglón 3). Adicionalmente, todos los fármacos mencionados en D3 (como ciclosporina, ritonavir, saquinavir y ontazolast) son altamente lipofílicos. Así, las enseñanzas de D3 concuerdan con las enseñanzas de D1 y no menciona ni sugiere la formulación de fármacos no lipofílicos en un SEDD. Tal como en D1, los fármacos considerados en D3 evidencian una baja solubilidad en agua (abstract, renglón 1). Sin embargo, no hay en dicho documento enseñanza o sugerencia alguna de que los fármacos no sean solubles en agua, así como el NRC-AN-019.

Contrario a lo anterior, la presente invención está relacionada con incrementar la biodisponibilidad del fármaco NRC-AN-019. Dicho compuesto es completamente diferente a los compuestos de piranona de D1 y a los compuestos de D3. En particular, mientras que D1 está restringido únicamente a compuestos que son lipofílicos y parcialmente

solubles en agua, el compuesto NRC-AN-019 de la presente invención no es lipofílico en lo absoluto y es completamente hidrofóbico en la naturaleza (no es soluble en agua).

Con respecto a lo anterior, comúnmente los compuestos que tienen solubilidad limitada en agua, como los de D1, tenderán a ser lipofílicos naturalmente y a tener buena solubilidad en solventes orgánicos no polares (y vice-versa). Sin embargo, esto no siempre se cumple. Así, existen compuestos que tienen buena solubilidad en solventes acuosos y solventes orgánicos no polares. También existen compuestos que tienen muy baja, o ninguna, solubilidad en ambos tipos de solventes; este es el caso del compuesto NRC-AN-019. Esto se evidencia en los datos experimentales reportados en la declaración de Durga Maheswari Parvataneni *et al* (adjunta):

- La declaración mencionada confirma que el compuesto NRC-AN-019 es completamente hidrofóbico en la naturaleza, siendo insoluble en agua. Por lo tanto, incluso después de 4 horas agitando el compuesto en agua, no se detectó disolución alguna de dicho compuesto (ver resultado en la página 3).
- Dicha declaración confirma que el compuesto NRC-AN-019 también tiene una baja solubilidad en los solventes orgánicos no polares 1-octanol, n-hexano, tolueno y ciclohexano. La solubilidad reportada está entre 0.22 y 535.48 µg/mL (ver página 4). Basándose en una densidad de 0.824 g/mL para el 1-octanol y 0.799 g/mL para el ciclohexano, esto significa que para disolver una parte de NRC-AN-019 se requerirían entre 1.500 y 3'500.000 partes de dichos solventes.
- La declaración confirma que el compuesto NRC-AN-019 también tiene una solubilidad baja en lípidos. La solubilidad de este compuesto está reportada entre 0.06 y 3.27 mg/mL (ver páginas 7 a 11). Esto significa que el número de partes de lípido por peso para disolver una parte de NRC-AN-019 está entre 300 y 15.000.
- De acuerdo con la nomenclatura mencionada en las páginas adjuntas de la Pharmacopoeia estadounidense, la solubilidad del compuesto NRC-AN-019 en solventes orgánicos no polares está entre "muy ligeramente soluble" y "prácticamente insoluble", y su solubilidad en lípidos está entre "ligeramente insoluble" y "prácticamente insoluble".

Todo lo anterior demuestra que el compuesto NRC-AN-019 es fundamentalmente diferente de los compuestos de piranona de D1 y de los compuestos lipofílicos de D3.

El señor examinador afirma que habría resultado obvio para una persona versada en la materia formular el compuesto NRC-AN-019 mediante los métodos o sistemas descritos en D1 y D3. Sin embargo, las diferencias evidenciadas entre el compuesto de la presente invención y los de los documentos D1 y D3, así como las enseñanzas del estado de la técnica, demuestran que una persona versada en la materia no habría tenido motivación alguna para realizar dicha formulación con la expectativa de obtener resultados positivos.

De todo lo anterior resulta evidente que la presente invención supera un prejuicio técnico, ya que va exactamente en contra de las enseñanzas de D1 y D3 pero obtiene resultados favorables. Esto corresponde a un indicio de la existencia de nivel inventivo, tal como se define en el numeral 2.13.5.3.1 de la "GUÍA PARA EXAMEN DE SOLICITUDES DE PATENTE DE INVENCION Y MODELO DE UTILIDAD" de su Despacho.

De acuerdo a todos los argumentos presentados, la presente solicitud cumple a cabalidad con los requerimientos exigidos en la Decisión 486 y las objeciones formuladas por el Examinador se ven superadas.

4. Puesto que se ha dado respuesta a todas las objeciones formuladas por el Examinador, se solicita atentamente proceder al otorgamiento de la solicitud de patente. En este sentido, respetuosamente recordamos al Examinador que la patente no podrá ser negada con base en objeciones diferentes a las expuestas en la acción oficial No. 13642, toda vez que al no haber sido trasladadas a mi representada, dicha decisión violaría tanto el tenor literal del artículo 45 de la Decisión 486 como el derecho constitucional al debido proceso. En este evento, y con base en el inciso 2 del artículo 45, respetuosamente solicitamos al Examinador expedir una nueva acción oficial que permita a mi representada contra argumentar las mismas o modificar la solicitud con el fin de cumplir los requisitos de patentabilidad.

5. **Anexos**

- Nuevo pliego reivindicatorio conformado por 26 reivindicaciones
- Comprobante de pago por 7 reivindicaciones adicionales después de las diecinueve reivindicaciones previamente pagadas, por un valor de \$ 224.000.
- Declaración de Durga Maheswari Parvataneni *et al* ante la USPTO y traducción de la misma.
- "Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs" de Christopher J.H. Porter *et al*.
- Extracto de la Pharmacopoeia estadounidense

Señor Director,


LUZ CLEMENCIA DE PÁEZ
C.C. 35.456.344 de Usaquén
T.P.A. No. 23.555

COLO-17766-0841-003-4
SML\SML\ID-277388\WPC17766
No. M-2015-277388\SML

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 -hidro- β -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 caprilcaproilo.
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 ó 9, 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.

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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-agente 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- β -hidroxipoli(oxi-1,2-etanodiilo), etanol o alcohol bencílico..

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800.176.089-2

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RECIBIDO DE : CAVELIER ABOGADOS

NI 860.041.367

RE

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR PAGO
RECIBO DE CA	999999999	10109	27/01/2015		109.000.00
RECIBO DE CA	999999999	10112	27/01/2015		119.000.00

*** Conceptos Pagados ***

CANT. RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
7 50005-01-01 SOLICITUDES	1607 REIVINDICACION PATENTE UNITARIA ADICIONAL A LAS 10 INIC	32.000.00	224.000.00
			<u>\$224.000.00</u>

SON: **DOSCIENTOS VEINTICUATRO MIL PESOS MONEDA CORRIENTE**

Responsable: _____

Recibo de Caja Aplicado al Expediente No. _____

0010-17766-0841-003-4

Sede Centro: Carrera 13 No. 27 - 00 Pisos 3,4,5 y 10 Bogotá, D.C. - Colombia

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, Enero 29 de 2015 - 16:32:09

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.

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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) 5 μ ;
Flow rate: 2.0 mL/min; Injection volume: 20 μ L; 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

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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;

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Flow rate: 2.0 mL/min; Injection volume: 20 μ L; 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 1 mL 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

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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).

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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) 5 μ ; Flow rate: 2.0 mL/min; Wave length: UV-270 nm; Column temperature: 40°C; Injection volume: 20 μ L; 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 1 mL 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.

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Procedure: Separately injected equal volumes (20 μ L) 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

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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 lipophile 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

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	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 + Com 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

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	(ADL/165)			
59	FRD/258/378 & AR/069/06/2011 (ADL/165)	API/L/513	NRC-AN-019 + Incroomega	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

EN LA OFICINA DE MARCAS Y PATENTES DE LOS ESTADOS UNIDOS

En referencia a la Solicitud de Patente de:
Durga Maheswari Parvataneni *et al*

No. de Solicitud:	14/003,445 (Fase nacional de PCT/IB2011/001842)	No. de Confirmación:	5330
Rad:	Agosto 10 de 2011	Unidad Técnica:	1629
De:	FORMULACIÓN QUE COMPRENDE UN DERIVADO DE FENILAMINOPIRIMIDINA COMO AGENTE ACTIVO	Examinador:	Lee, William Y

DECLARACIÓN DE CONFORMIDAD CON 37 C.F.R. § 1.132

Declaro que:

1. Soy Gerente Senior del Desarrollo e Investigación de Formulaciones en Natco Pharma Limited.
2. He completado un Master en Farmacia en la Facultad de Ciencias Farmacéuticas de la Universidad de Andhra, Visakhapatnam, Andhra Pradesh y actualmente estoy cursando un doctorado titulado “Formulaciones de fármacos poco solubles en agua que utilizan la tecnología de solubilización micelar” de la Universidad Tecnológica de Jawaharlal Nehru, Hyderabad, India. Tengo 15 años de experiencia en actividades de investigación, he publicado 4 artículos de investigación y he orientado a 18 estudiantes del Master de Farmacia en sus proyectos de investigación. He trabajado como Gerente Senior del Desarrollo e Investigación de Formulaciones, Natco Pharma Limited, Hyderabad, India desde mayo de 2003 y antes de esto trabajé como químico y Subgerente de R&D, en Tablets (India) Limited, Chennai, India.
3. He leído y entendido (i) la solicitud de patente de EE.UU. 14/003,445.
4. Se realizó un experimento para determinar **la solubilidad de NRC-AN-019 en un disolvente polar (Agua)**. El procedimiento fue el siguiente. Se llevaron a cabo experimentos del balance de la solubilidad en un matraz aforado o matraz cónico de 250 ml, conservado a una temperatura ambiente. Se transfirió una muestra sólida de la parte conocida a un matraz cónico de 250 ml que contenía 50 ml de disolvente polar, y se agitó a 200 rpm en un agitador rotatorio. Se filtraron, se diluyeron, se inyectaron y se tomaron alícuotas a intervalos regulares en el sistema de HPLC.

Las Condiciones de Solubilidad fueron: Medio: Agua; Volumen; 50 ml; RPM: 200;

Temperatura: Temperatura ambiente; y Tiempos: Alrededor de 1, 2, y 4 horas.

Las Condiciones Cromatográficas fueron: Columna: Purospher star RP 18e (150 x 4.6 mm) 5 μ ; Velocidad de flujo: 2.0 ml/min; Volumen de inyección: 20 μ L; Detección: 270 nm; Temperatura de la Columna del Horno: 40°C; y Tiempo de Ejecución: 14 minutos.

Las preparaciones fueron las siguientes.

Preparación de las Soluciones Tampón de pH 3.0: Se disolvió 1,36 g de dihidrogenofosfato de potasio en 1000 ml de agua milli-Q, y se ajustó el pH a 3,0 con ácido ortofosfórico y se filtró a través de un filtro de 0,45 μ .

Preparación de la fase móvil: Se mezcló el tampón y el acetonitrilo de pH 3,0 en la proporción de 45:55 v/v y se des gasificó durante 10 minutos.

Diluyente: Se mezcló el agua y el acetonitrilo en la proporción de 5:96 v/v y se des gasificó durante 10 minutos.

Preparación estándar: Se pesó y se transfirió exactamente alrededor de 50 mg de NRC-AN-019 (patrón de trabajo) en un matraz aforado de 100 ml, se añadió 1 ml de dimetilformamida, se remolino y se añadió alrededor de 70 ml de metanol, la solución se disolvió de forma sónica, se dejó enfriar a temperatura ambiente y se diluyó a volumen con metanol. De la pipeta se sacaron 5 ml de la solución anterior en un matraz aforado de 50 ml y se diluyó a volumen con diluyente. Después se diluyeron 2 ml de la solución anterior a 50 ml con diluyentes (2 ppm).

Preparación de prueba: Se pesó y transfirió exactamente alrededor de 10 mg de NRC-AN-019 en un matraz cónico de 250 ml, se añadieron 50 ml de medio, se remolino y se observó la solubilidad. Si se encontraba algo de solubilidad, se verificaba añadiendo otros 10 mg de NRC-AN-019, se remolinaba, se observa la solubilidad y se repetía el procedimiento hasta que alguna muestra permaneciera insoluble. Se continuaba con la agitación en un agitador rotatorio durante una hora y se observa la solubilidad. Si se encontraba solubilidad, se añadía otra porción (10 mg), se agitaba durante una hora y luego se repetía el procedimiento hasta que algún muestra permaneciera insoluble. Se continuaba con la agitación durante 4 horas y se recogían 5 ml de la solución de muestra a intervalos regulares, es decir, de una hora, de dos horas y de cuatro horas. La solución de muestra se filtraba y se desechaba una parte, se recogía el restante y se diluía a una concentración que está en el intervalo lineal de concentración estándar y se inyectaba en el sistema de HPLC.

Resultado: La solubilidad del NRC-AN-019 en agua: **ninguna solubilidad incluso después de la cuarta hora.**

5. Se realizó un experimento para determinar **la solubilidad de NRC-AN-019 en disolventes no polares (1-Octanol, n-Hexano, Tolueno y ciclohexano)**. El procedimiento fue el siguiente. Se llevaron a cabo experimentos del balance de la solubilidad en un matraz aforado o matraz cónico de 250 ml, conservado a una temperatura ambiente. Se transfirió una muestra sólida de la parte conocida a un matraz cónico de 250 ml que contenía 50 ml de disolvente no polar, y se agitó a 200 rpm en un agitador rotatorio. Se filtraron, se diluyeron, se inyectaron y se tomaron alícuotas a intervalos regulares en el sistema de HPLC.

Las Condiciones de Solubilidad fueron: Medio: 1-Octanol, n-Hexano, Tolueno y ciclohexano; Volumen: 50 ml; RPM: 200; Temperatura: Temperatura ambiente; y los Tiempos: Alrededor de 1, 2, y 4 horas.

Las Condiciones Cromatográficas fueron: Columna: Purospher star RP 18e (150 x 4.6 mm) 5 μ ;

Velocidad de flujo: 2.0 ml/min; Volumen de inyección: 20 μ L; Detección: 270 nm; Temperatura de la Columna del Horno: 40°C; y Tempo de Ejecución: 14 minutos.

Las preparaciones fueron las siguientes.

Preparación de las Soluciones Tampón de pH 3.0: Se disolvió 1,36 g de dihidrogenofosfato de potasio en 1000 ml de agua milli-Q, y se ajustó el pH a 3,0 con ácido ortofosfórico y se filtró a través de un filtro de 0,45 μ .

Preparación de la fase móvil: Se mezcló el tampón y el acetonitrilo de pH 3,0 en la proporción de 45,55 v/v y se des gasificó durante 10 minutos.

Diluyente: Se mezcló el agua y el acetonitrilo en la proporción de 5:95 v/v y se des gasificó durante 10 minutos.

Preparación estándar: Se pesó y se transfirió exactamente alrededor de 50 mg de NRC-AN-019 (patrón de trabajo) en un matraz aforado de 100 ml, se añadió 1 ml de dimetilformamida, se remolino y se añadió alrededor de 70 ml de metanol, la solución se disolvió de forma sónica, se dejó enfriar a temperatura ambiente y se diluyó a volumen con metanol. De la pipeta se sacaron 5 ml de la solución anterior en un matraz aforado de 50 ml y se diluyó a volumen con diluyente. Después se diluyeron 2 ml de la solución anterior a 50 ml con diluyentes (2 ppm).

Preparación de prueba: Se pesó y se transfirió exactamente alrededor de 10 mg de NRC-AN-019 en un matraz cónico de 250 ml, se añadió 50 ml de medio, se remolino y se observó la solubilidad. Si se encontraba algo de solubilidad, se verificaba añadiendo otros 10 mg de NRC-AN-019, se remolinaba, se observa la solubilidad y se repetía el procedimiento hasta que alguna muestra permaneciera insoluble. Se

continuaba con la agitación en un agitador rotatorio durante una hora y se observa la solubilidad. Si se encontraba solubilidad, se añadía otra porción (10 mg), se agitaba durante una hora y luego se repetía el procedimiento hasta que algún muestra permaneciera insoluble. Se continuaba la agitación durante 4 horas y se recogían 5 ml de solución de muestra a intervalos regulares, es decir, de una hora, de dos horas y de cuatro horas. La solución de muestra se filtraba y se desechara una parte, se recogía el restante y se diluía a una concentración que está en el intervalo lineal de concentración estándar y se inyectaba en el sistema de HPLC.

Resultado: La solubilidad de NRC-AN-019 en los disolventes no polares evaluados fue la siguiente.

- a) **1-Octanol: 535.48 µg/ml a la cuarta hora.**
 - b) **N-Hexano: 0.86 µg/ml a la cuarta hora.**
 - c) **Tolueno: 13.20 µg/ml a la cuarta hora.**
 - d) **Ciclohexano: 0.22 µg/ml a la cuarta hora.**
6. Se realizó un experimento para determinar **la solubilidad de NRC-AN-019 en diferentes lípidos).**

Nota: Dado que los excipientes tienen una solubilidad en diferentes diluyentes, fue imposible establecer un diluyente común y condiciones cromatográficas. Por lo tanto, la solubilidad de NRC-AN-019 se determinó en fase normal, así como en condiciones de fase inversa usando diferentes diluyentes y condiciones cromatográficas.

Fase normal de Condiciones cromatográficas: Columna: Inertsil Silica (250 mm x 4.6 mm) 5 µ; Velocidad de flujo: 1.0 ml/min; Volumen de inyección: 50 µL; Detección: 270 nm; Temperatura de la Columna: 30°C; y Tempo de Ejecución: 12 minutos.

Preparación de la fase móvil: Se mezcló n-Hexano, alcohol Isopropílico y metanol en la proporción de 70:20:10 v/v, respectivamente, y se desgasificó durante 5 minutos.

Preparación estándar: Se pesó y se transfirió exactamente alrededor de 50 mg de NRC-AN-019 (patrón de trabajo) en un matraz aforado de 100 ml, se añadió 70 ml de metanol, la solución se disolvió de forma sónica, se dejó enfriar a temperatura ambiente y se diluyó a volumen con metanol. Se transfirieron 4 ml de la solución anterior a 100 ml y se completó el volumen con diluyentes (20 ppm).

Preparación de prueba: Se pesaron alrededor de 1200 mg de la muestra en un tubo eppendorf y se mantuvo en centrifugación a 10000 rpm durante 30 minutos. Luego, se pesaron alrededor de 300 mg de centrifugado en un matraz aforado de 50 ml y se diluyó a volumen utilizando un disolvente o una mezcla, hasta que se obtuvo una solución clara. De ser necesario se diluía adecuadamente a una concentración, la cual

está en el intervalo lineal de concentración estándar, y se inyectaba en el sistema de HPLC.

Fase inversa de Condiciones cromatográficas: Columna: Purospher star RP 18e (150 x 4.6 mm) 5 μ ; Velocidad de flujo: 2.0 ml/min; Longitud de onda: UV-270 nm; Temperatura de la columna: 40°C; Volumen de inyección: 20 μ L; y Tiempo de Ejecución: 14 minutos.

Preparación del tampón: Se pesó y se disolvió alrededor de 1,36 g de dihidrogenofosfato de potasio en 1000 ml de agua Milli-Q y se ajustó el pH a 3,0 con ácido ortofosfórico, se filtró la solución tampón a través de un filtro de membrana de 0,45 μ .

Preparación de fase móvil: Se mezcló el tampón y el acetonitrilo de pH 3,0 en la proporción de 45:55 v/v, respectivamente, y la mezcla se des gasificó durante 5 minutos.

Preparación estándar: Se pesó y se transfirió exactamente alrededor de 100 mg de NRC-AN-019 (patrón de trabajo) en un matraz aforado de 100 ml, se añadió 1 ml de dimetilformamida, se remolinaron los contenidos hasta disolverse, se añadió alrededor de 70 ml de metanol, la solución se disolvió de forma sónica, se dejó enfriar a temperatura ambiente y se diluyó a volumen con metanol. Se transfirieron 4 ml de la solución anterior a un matraz aforado de 100 ml y se diluyó con un diluyente (40 ppm).

Preparación de prueba: Se pesaron alrededor de 1200 mg de la muestra en un tubo eppendorf y se mantuvo en centrifugación a 10000 rpm durante 30 minutos. Luego, se pesaron alrededor de 300 mg del centrifugado en un matraz aforado de 50 ml y se diluyó a volumen utilizando un disolvente o una mezcla, hasta que se obtuvo una solución clara. De ser necesario se diluía adecuadamente a una concentración, la cual está en el intervalo lineal de concentración estándar, y se inyectaba en el sistema de HPLC.

Procedimiento: Los volúmenes iguales (20 μ L) de los análisis preliminares y de la preparación estándar (seis inyecciones) y de las preparaciones de muestra en el cromatógrafo se inyectaron por separado, y se registraron los cromatogramas.

Aplicabilidad del sistema: Se tomaron los cromatogramas de la preparación estándar (seis inyecciones), se midió el área del pico para el pico de analito y se evaluaron los parámetros de idoneidad del sistema. Los parámetros de idoneidad del sistema cumplieron con los siguientes criterios de aceptación: (i) el % de RSD para las seis inyecciones de área de pico de NRC-AN-019 a partir de la preparación estándar no debe ser mayor de 2,0; (ii) el factor de asimetría para NRC-AN-019 a partir de la preparación estándar no debe ser mayor de 2,0; y (iii) el contenido de gérmenes para

NRC-AN-019 a partir de la preparación estándar no debe ser inferior a 2000.

Resultados:

No. de muestra	B. No. & Exp No.	Código de la muestra	Nombre de la muestra	Solubilidad (mg/ml)
1	FRD/171/029-01 & 019-LBF-040 (ADL/115)	API/L/01	NRC-AN-019 + Ácido oleico	1,32
2		API/L/02	NRC-AN-019 + MCT	0,22
3		API/L/03	NRC-AN-019 + Aceite de oliva	0,06
4		API/L/04	NRC-AN-019 + Aceite de girasol	0,09
5		API/L/05	NRC-AN-019 + Aceite de ricino	0,40
6		API/L/06	NRC-AN-019 + Aceite de cacahuete	0,12
7		API/L/07	NRC-AN-019 + Aceite de coco	0,12
8		API/L/08	NRC-AN-019 + Aceite de soya	0,06
9		API/L/09	NRC-AN-019 + Aceite de palma	0,09
10		API/L/10	NRC-AN-019 + Aceite de sésamo	0,12
11	FRD/171/030 & 019-LBF-041 (ADL/115)	API/L/11	NRC-AN-019 + Aceite de mostaza	0,09
12	FRD/171/032 & 019-LBF-042 (ADL/115)		NRC-AN-019 + Lauroglicol	1,16
13			NRC-AN-019 + Labrafac	0,33
14			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 + Aceite de canola	0,06
21	FRD/171/037 & 019-LBF-048 (ADL/115)	API/L/82	NRC-AN-019 + Aceite de almendras	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 + Oleato de etilo	0,14
27	FRD/196/114 & 019-LBF-099 (ADL/120)	API/L/307	NRC-AN-019 + Oleilalcohol	0,44
28	FRD/210/119 & 019-LBF-118 (ADL/128)	API/L/320	NRC-AN-019 + Aceite de maíz, refinado y puro	0,09
29		API/L/321	NRC-AN-019 + Labrafac lipofile WL 1349	0,23
30		API/L/322	NRC-AN-019 + Lauroglicol FCC	0,60
31		API/L/328	NRC-AN-019 + Capmul GMO-50	0,30
32	FRD/228/173 & 019-	API/L/395	NRC-AN-019 + Labrafil M 1944 CS	0,53

33	LBF-234 (ADL/135)	API/L/396	NRC-AN-019 + Plurol Olique CC 497	0,29
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 + Ácido ricinoleico	3,24
37		API/L/416	NRC-AN-019 + Aceite de linaza	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 + Caproil 90	2,19
40		API/L/429	NRC-AN-019 + Caprol MPGO	0,65
41		API/L/431	NRC-AN-019 + Parafina líquida	0,17
42		API/L/432	NRC-AN-019 + Mezcla de Aceite de maíz con Aceite de Girasol	0,19
43	FRD/246/295-02 & R/056/02/2011 (ADL/159)	API/L/441	NRC-AN-019 + Aceite de linaza	0,10
44		API/L/442	NRC-AN-019 + Ácido linoleico	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 + Lipoidea 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	FRD/246/327 & AR/086/04/2011 (ADL/162)	API/L/465	NRC-AN-019 + Capmul MCM	1,19
51		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 + Ácido linoleico	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 (ADL/165)	API/L/507	NRC-AN-019 + Aceite de hígado de bacalao	0,06
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 + Oleato de etilo	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 + Lauroglicol 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 + Lauroglicol-90	0,81
65		API/L/564	NRC-AN-019 + Caproil 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	FRD/277/472 &	API/L/567	NRC-AN-019 + Captex 200P	0,24
69		API/L/574	NRC-AN-019 + Caprol 3GO	0,31

70	AR/062/09/2011 (ADL/174)	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

Fecha: 22-10-2014

Durga Maheswari Parvataneni

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

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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.

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 screens¹.

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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doi: 10.1038/nrd2197

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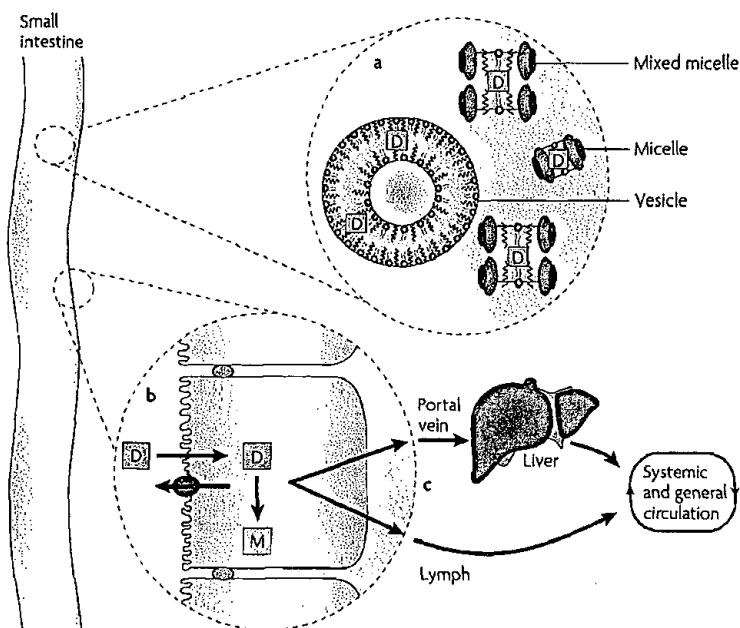


Figure 1 | Potential effect of lipids and lipidic excipients on drug absorption. Lipids can affect drug absorption in three ways: by enhancing drug (D) solubilization in the intestinal milieu through alterations to the composition and character of the colloidal environment — for example, vesicles, mixed 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.

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 lipid² 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 pancreas⁸. 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 interface¹³. 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 bile¹⁴ 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

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.

Table 1 | Fasting intestinal bile salt and phospholipid concentrations

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

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 species³⁰. 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 C₁₈ lipids) vary significantly when compared to medium-chain triglycerides³⁴ (BOX 2). C₁₈ fatty acids and monoglycerides are considerably less polar than their C₈ or C₁₂ 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

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 16:4:1.

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.

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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 ^a	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	Jejunum	213
15.8 ± 5.6* (mean ± SD, n = 5)	NR	0.5–1 h	Meal supplemented with long-chain triglyceride, 48% carbohydrate, 36% fat, 16% protein, total 400 Kcal	Jejunum	214
6.8 ± 1.72* (mean ± SD, n = 5)	NR	0.5–1 h	Meal supplemented with medium-chain triglyceride, 48% carbohydrate, 36% fat, 16% protein, total 400 Kcal	Jejunum	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	Jejunum	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 loads³⁶, but is significantly reduced as the mass of exogenous lipid in the digest is decreased³³, and that digestion of certain surfactants might lead to significant decreases in solubilization capacity for some formulations³⁹. By contrast, in the presence of long-chain triglycerides, effective drug solubilization is often possible even at low lipid concentrations³⁵. 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^{33,35,38,40}.

Although the data from our laboratories (and others), which describe trends in solubilization and oral bioavailability for highly lipophilic drugs such as halofantrine, cinnarizine³³ and cyclosporine⁴¹, 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 pendeclonidine^{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.

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

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.

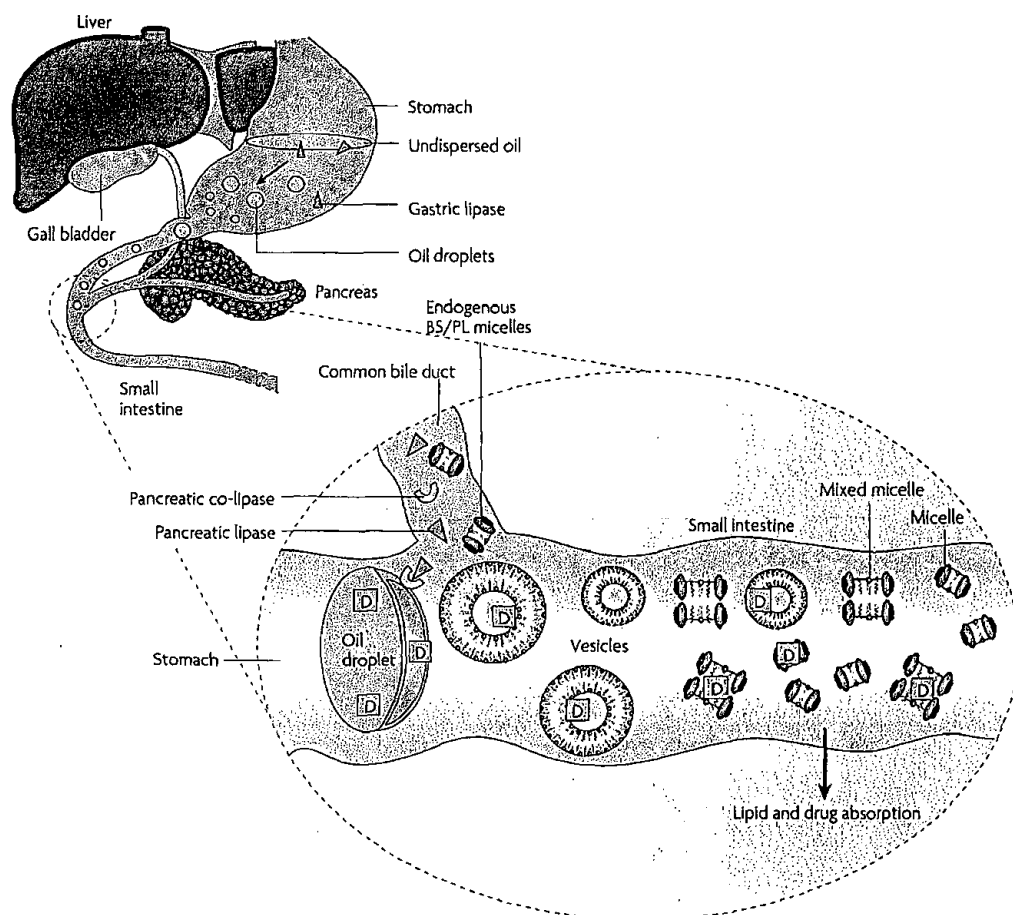


Figure 2 | Lipid digestion and drug solubilization in the small intestine. Following ingestion, the digestion of exogenous dietary triglyceride (TG) and formulation TG is initiated in the stomach by gastric lipase. The stomach further contributes to lipid processing by mechanical mixing (propulsion, 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-lipase²⁰³ 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 A₂ 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 cholesterol 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.

Intestinal lymphatic drug transport

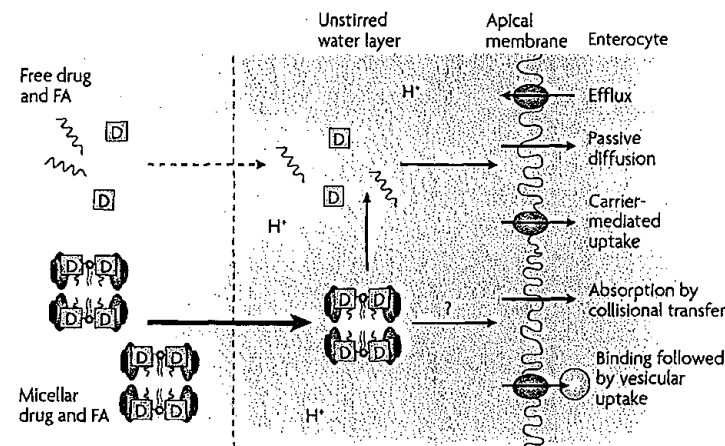
The intestinal lymphatic system (BOX 4) is a unique, high-capacity drug transport pathway, which has the potential to enhance the exposure of co-administered lipophilic drugs. Currently, only a few drugs that are in clinical use are substantially transported to the systemic circulation by the intestinal 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 triglycerides²⁵. 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

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)²⁶. 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 microclimate²⁶. 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, MG²¹⁸ and lipophilic molecules²¹⁹ 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^{26,218} 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.



Chylomicrons
Chylomicrons are larger (50–500 nm) and less dense ($S_0 > 400$) 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 hydrocarbons⁵⁵ and halofantrine⁵⁶ 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 lipid⁵⁷. 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 moxidectin⁵⁸, halofantrine^{43,59,60}, mepitiostane^{61–63}, testosterone derivatives⁶⁴, MK-386 (a 5 α -reductase inhibitor)⁶⁵, penclomedine⁶², naftifine⁶⁶, probucol⁶⁷, cyclosporine⁶⁸, ontazolast⁶⁹, CI-976 (REF. 70), fat-soluble vitamins and their derivatives, retinoids⁷¹, lycopene⁷², DDT and analogues^{54,57}, benzopyrene, and PCBs (polychlorinated biphenyls)⁵⁷.

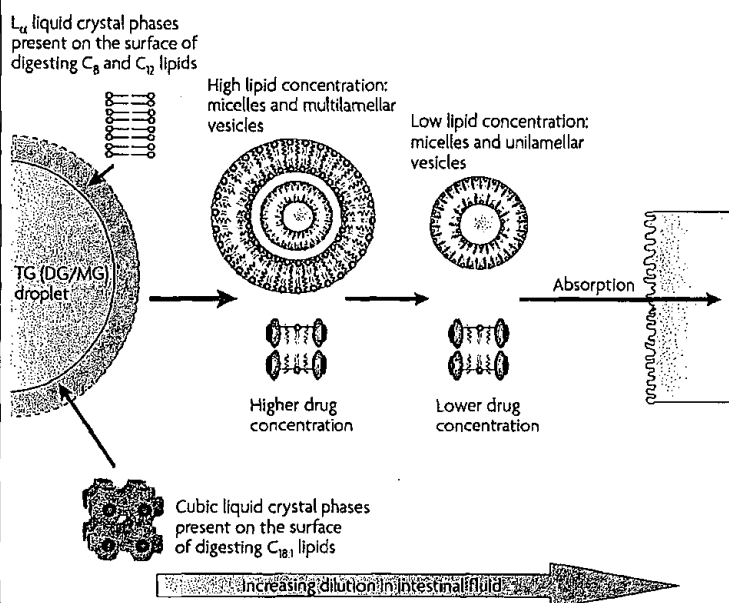
Implications of lymphatic drug transport

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, testosterone⁶⁴ (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 metabolism⁷³.

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 tissue⁷⁵. It is possible therefore that increasing the concentration of anti-HIV and anticancer compounds in the mesenteric lymph might improve therapy.

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^{217,227,228}. More recently, pseudo-tertiary phase diagrams have also been used to characterize and map the potential post-digestion phase behaviour of selected formulation lipids³⁰. 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_u) 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_u 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*.



REVIEWS

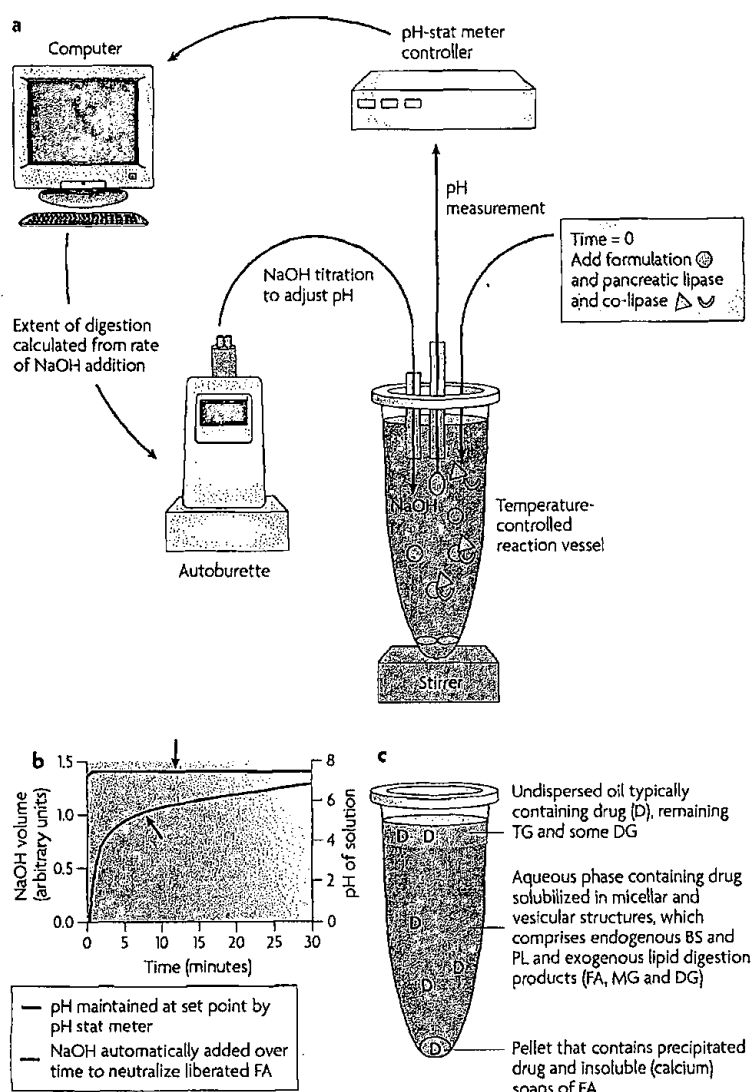


Figure 3 | Lipid digestion models for *in vitro* assessment of lipidic formulations. Lipid digestion models are being increasingly used as tools to facilitate improved *in vitro* evaluation of lipid-based drug delivery systems. Although the experimental details of these models differ slightly between 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 digest 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 conditions⁷⁶. More recently, a thoracic lymph-duct cannulated dog model has also been described^{79,77}, 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

intestine). The pig might also provide an alternative to the dog as a suitable larger animal model^{76,244}. 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

colchicine⁸¹. 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 assembly⁵². 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.

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, respectively⁵⁹. 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 administration⁸⁶. Interestingly, the mass of triglyceride recovered in the lymph over the 10 hours also suggested that administration of a small amount of exogenously

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.

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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

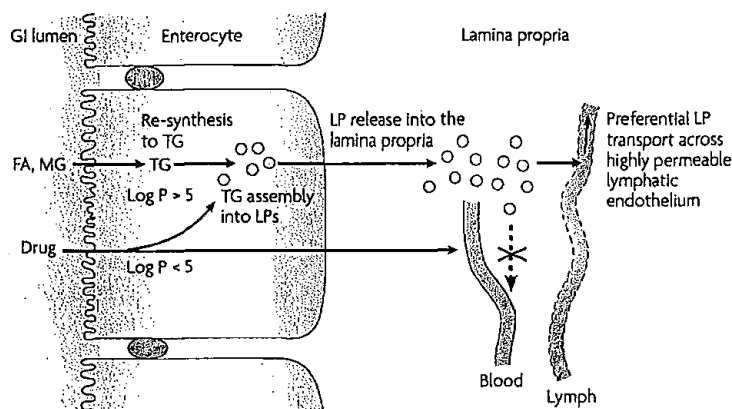


Figure 4 | Lipid and drug transport by the mesenteric lymph or portal blood following oral delivery. Following uptake into the enterocytes, lipid digestion products, for example fatty acid (FA) and monoglyceride (MG), can be re-synthesized to triglyceride (TG) in the smooth endoplasmic reticulum and 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.

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 blood⁹¹⁻⁹³. Predictably, therefore, the use of long-chain fatty acids has been shown to significantly increase drug transport into the lymph⁴³. In general, monounsaturated and polyunsaturated fatty acids also promote lymphatic lipid transport more readily and produce larger lipoproteins when compared with saturated fatty acids⁹⁴⁻¹⁰⁰.

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^{40,63,101,102}. 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 halofantrine¹⁰³ and α -tocopherol¹⁰⁴ 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 halofantrine¹⁰⁵, CI-976 (REF. 70), mepitiostane⁶³, cyclosporine¹⁰⁶, D - α -tocopherol acetate¹⁰⁷, retinol, and retinyl palmitate¹⁰⁸. 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^{160,105}. 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 drug¹⁰⁹. 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}.

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 humans⁶⁴. 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 5 α -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 undecanoate⁶⁴. 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 transport¹¹⁶ (FIG. 5). Uptake is believed to be largely carrier-dependent at low lipid concentrations and primarily passive at higher fatty-acid concentrations¹¹⁵. 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 BI¹²², GP330 (also known as low-density lipoprotein-related protein 2)¹²³ and caveolin¹²⁴. 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}, caveolin¹²⁴, CD36 and FAT¹¹⁷, 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 oligopeptides¹³¹, monosaccharides¹³², organic cations¹³³, organic anions¹³⁴, and bile acids¹³⁵ 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 receptors¹³⁸. 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 bile¹⁴³ 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 secretion⁸³. 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 proteins¹⁵¹, and ileal bile-acid-binding protein (I-BABP)¹⁴⁸. It has been suggested that lipid-binding proteins might facilitate the diffusion of lipids across enterocytes¹⁵² and/or act as cytosolic storage 'sinks' for excess, and potentially toxic, quantities of free fatty-acid and exogenous substances¹⁵³. 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 ($S_0 = 20–400$) 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 $D_{7.4}$

Log₁₀ 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.

REVIEWS

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 transport¹⁸⁴. These orphan nuclear receptors (and some known ligands) include: the peroxisome proliferator-activated receptor (PPAR) for fatty acids²³²; liver X receptor (LXR) for oxysterols^{232,234}; farnesoid X receptor (FXR) for bile acids²³⁴; steroid and xenobiotic receptor (SXR) and pregnane xenobiotic receptor (PXR) for steroids and xenobiotics²³⁵; and constitutive androstane receptor (CAR) for xenobiotics²³⁶. PPAR α , PPAR δ and PPAR γ 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 metabolism²³². Specifically, PPAR α is believed to function as a global regulator of fatty-acid metabolism and catabolism. Ligands for PPAR α include indomethacin, LTD4 antagonists (for example, LY171993), and fibrate drugs (GW2331, clofibrate acid and fenofibrate). Natural ligands for PPAR α include fatty acid (both medium-chain and long-chain unsaturated) and eicosanoids and their metabolites. PPAR γ 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 receptor²³². The ligands for PPAR γ also include indomethacin, LTD4 antagonists and fibrates (although the fibrates are weaker ligands for PPAR γ than for PPAR α), and natural ligands for PPAR γ include arachidonic acid metabolites, fatty acid and triterpenoids. PPAR δ has a less well understood function^{237–239} and ligands include essential long-chain fatty acids, iloprost, carbaprostacyclin and some fibrates. PPAR δ agonists seem to induce liver fatty-acid-binding protein (L-FABP) in the small intestine whereas PPAR α seems to regulate L-FABP expression in the liver¹⁸². 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 enterocyte¹⁵⁴.

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-gp¹⁷⁰. 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 humans¹⁷¹. 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-gp¹⁷³ 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 efflux¹⁷⁶. 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 transporters¹³⁵ 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-gp¹⁸⁰. 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 complex¹⁸⁴. By contrast, L-FABP levels are reduced following presentation of a chronic low-fat diet to rats¹⁴⁷. 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 L-FABP and L-FABP in the small intestine of rats¹⁵⁴. As FABPs have been shown to bind to non-endogenous ligands, and in normal usage few formulations are administered as a

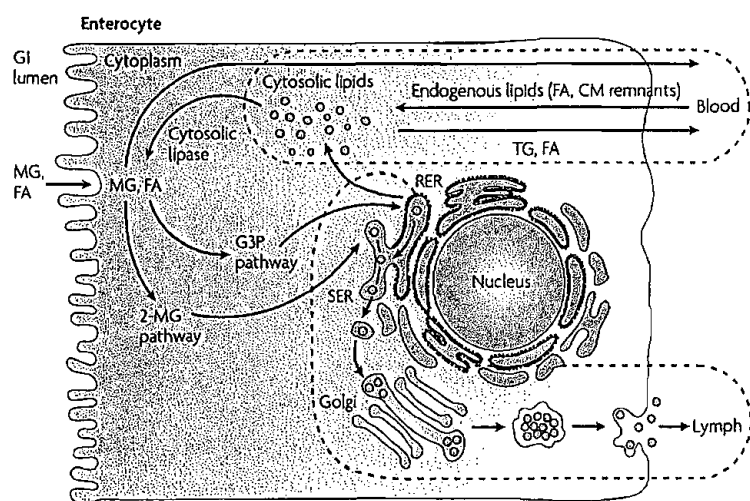


Figure 5 | Pathways of lipid absorption and pooling within the enterocyte. Following uptake across the apical membrane of the enterocyte, the products of gastrointestinal (GI) lumen lipid digestion — monoglyceride (MG) and fatty acid (FA) — can either diffuse across the enterocyte and enter the portal vein blood⁸⁹ 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 system²⁰⁷. 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 pathway¹⁹⁸ 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 lipase²⁰⁰ and the digestion products formed can be re-circulated into TG assembly pathways¹⁹⁵. 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 drugs¹⁰³.

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 enterocytes¹⁸⁷. 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 triglyceride 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 SER¹⁹⁵. 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 lipase²⁰⁰. 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^{198,199}. 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 pool¹⁰³ 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 pool²⁰¹. 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 inter-related. 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 transport²⁰¹.

REVIEWS

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^{190,240}. 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^{241,246}, 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 Golgi²⁴².

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 pools²⁰¹. 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 transport¹⁰³. 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 transport¹¹³. 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 metabolism⁷³. 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

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.

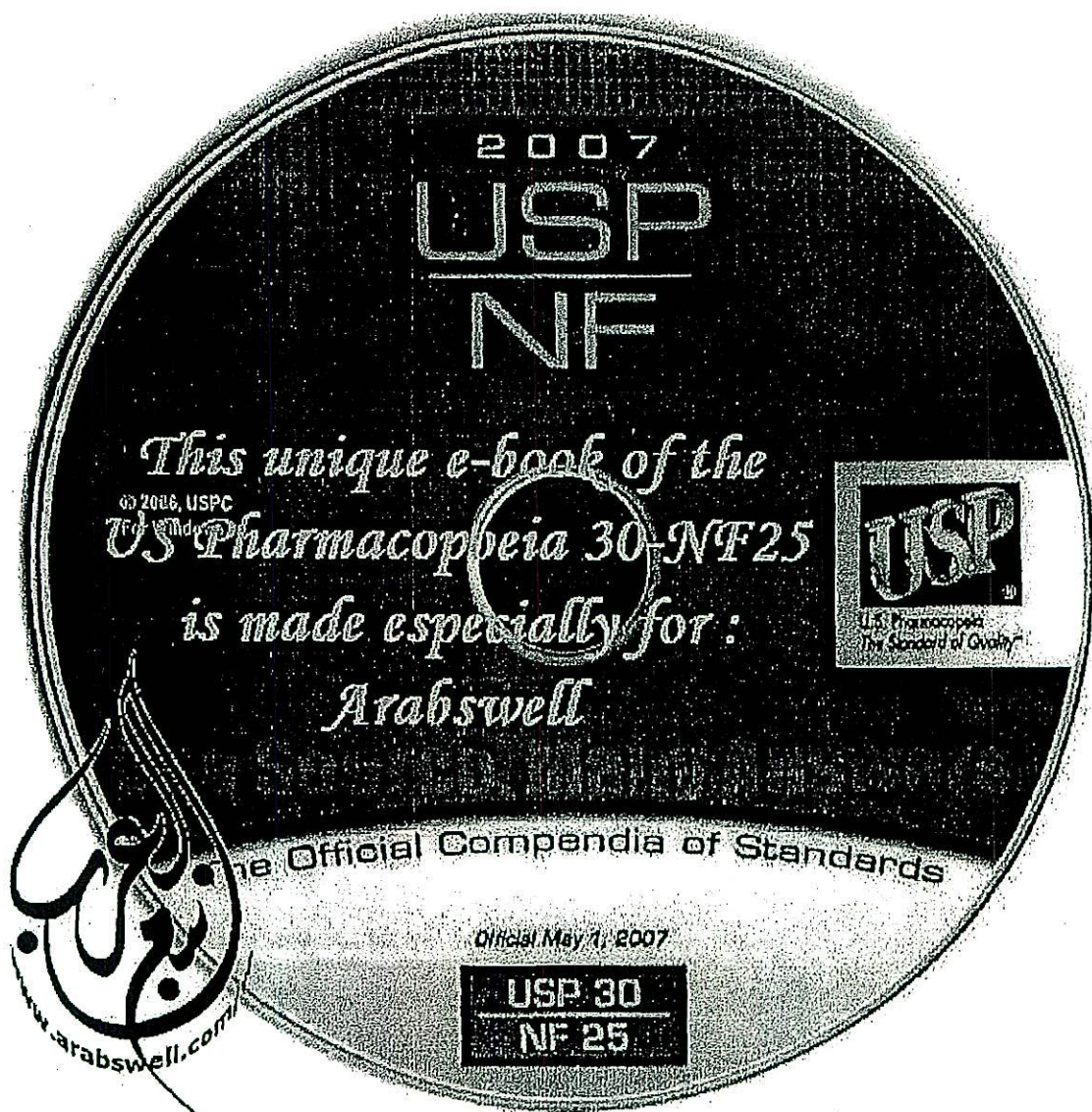
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Annexure : 4

Introduction

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Best Regards,
Lawrence

DESCRIPTION AND SOLUBILITY

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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 Pharmacopelial and National Formulary substances are indicated by the descriptive terms in the accompanying table. The term "miscible" as used in this Pharmacopelia 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 Pharmacopelial 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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