

Clarke, Modet & Cº

COLOMBIA

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SUPERINTENDENCIA DE INDUSTRIA Y CC
DIVISION DE NUEVAS CREACIONES

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Re: Expediente No. 13-252.771

Solicitud de patente a nombre de **CELLRESIN TECHNOLOGIES, LLC**

Yo, CAROLINA DAZA MONTALVO identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 No. 86-53, piso 6, obrando como apoderada sustituta de acuerdo con la sustitución adjunta de la sociedad **CELLRESIN TECHNOLOGIES, LLC**, domiciliada en Estados Unidos de América, solicitante de la patente de la referencia, en respuesta al oficio No. 1250 notificado por fijación en lista el 10 de febrero de 2015, relacionado con concepto técnico de fondo, me permito manifestar:

1. Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial nuevo capítulo reivindicatorio, el cual consta de 23 reivindicaciones y reemplaza completamente el capítulo presentado anteriormente.
2. Claridad
 - 2.1 Respecto al requerimiento del examinador solicitando una modificación al título de la presente solicitud, se solicita amablemente que dicho título sea reemplazado por: "COMPOSICIÓN DE CICLODEXTRINA, MATERIAL DE ENVASADO Y RECIPIENTE".
 - 2.2 Como primera medida, se modificaron las antiguas reivindicaciones 1 y 21 (nuevas reivindicaciones 1 y 20) incluyendo las características de la antigua reivindicación 2, con el fin de dar claridad a los monómeros polimerizables con radiación de la composición reclamada.
 - 2.3 Además se incluyó el rango "entre 0.001%p y un 25%p" del complejo de inclusión ciclodextrínico en las nuevas reivindicaciones 1, 7, 13 y 23 con el fin de dar claridad a la invención reclamada. Esta modificación se encuentra sustentada en la Página 19 del capítulo descriptivo.

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- 2.4 Se modificó el rango del complejo de inclusión ciclodextrínico de la antigua reivindicación 21 (nueva reivindicación 20). Esta modificación se encuentra sustentada en la Página 19 del capítulo descriptivo.
- 2.5 Se eliminó el término “aproximadamente” de las antiguas reivindicaciones 7 y 18 a 23 (nuevas reivindicaciones 6 y 17 a 22).
- 2.6 Con respecto a la antigua reivindicación 24 (nueva reivindicación 20), la solicitante desea manifestar respetuosamente que en dicha reivindicación se están especificando los compuestos reclamados. Por lo tanto, solicita amablemente se mantenga como una reivindicación independiente.
- 2.7 Se ha eliminado la expresión “y opcionalmente uno o más capturadores de agua, desecantes, promotores de la adhesión, agentes antiincrustantes, estabilizadores térmicos, estabilizadores oxidativos, colorantes, adyuvantes, plasticidas o mezclas de estos” del capítulo reivindicatorio.
- 2.8 El señor examinador indica que es un *“error caracterizar las otras reivindicaciones independientes a partir de las características de la composición de ciclodextrina”*. Al respecto, la solicitante respetuosamente opina que la composición de ciclodextrina es una característica esencial de las otras reivindicaciones independientes (del material de envasado, del recipiente, del método para preparar un material de envasado tratado y de la composición de ciclodextrina curada) y que por tanto no debe eliminarse dicha característica. Además, esta es la característica inventiva común entre las reivindicaciones independientes.

En cuanto a estas modificaciones, me permito decir que las mismas no amplían de ninguna manera la materia objeto de la presente solicitud y se realizaron con el ánimo de superar las objeciones de claridad y nivel inventivo planteadas por el señor examinador en su concepto técnico.

En consecuencia, nos permitimos informar que las nuevas reivindicaciones 1 a 23 se ajustan a las disposiciones del artículo 30 de la Decisión 486.

En cuanto al número de reivindicaciones, atentamente nos permitimos decir que no se adjunta tasa por reivindicaciones adicionales, toda vez que el nuevo capítulo reivindicatorio tiene menor número de cláusulas que el originalmente presentado y en esencia es el mismo objeto pero más limitado.

3. Unidad de Invención

Con respecto a lo establecido en la acción oficial, en donde el señor examinador indica que la presente invención se refiere a 4 grupos inventivos, es deseo de la Solicitante manifestar respetuosamente su desacuerdo con dicha objeción ya que las 4 invenciones a las que hace referencia el señor examinador se refieren a un concepto inventivo común, la composición de ciclodextrina, que es una composición nueva e inventiva.

La solicitante respetuosamente opina que las 4 invenciones están ligadas por un concepto inventivo común y que por tanto la presente solicitud tiene unidad de invención.

4. Nivel Inventivo

El señor examinador establece en su concepto técnico que las antiguas reivindicaciones 1 a 7 carecen de nivel inventivo a la luz de los documentos US 6,548,448 (D1) y US 6,162,533 (D2).

Además, el señor examinador establece que los grupos inventivos 2 a 4 carecen de nivel inventivo a la luz de los documentos US 6,548,448 (D1), US 6,162,533 (D2) y la combinación de D1 con el documento EP 1593306.

En este sentido, me permito decir que el artículo 18 de la Decisión 486, el cual hace referencia al nivel inventivo de una invención, establece que *“Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica”*.

Lo anterior significa que el análisis de nivel inventivo **NO** es una comparación de los objetos de las anterioridades y la solicitud en estudio. El análisis de nivel inventivo **debe demostrar** la obviedad de la invención y establecer cómo ésta no contiene un salto cualitativo, que demuestre su altura inventiva, teniendo en cuenta que se hace **necesario demostrar** que la invención analizada carece de nivel inventivo y hubiese sido obvia para una persona versada en la materia.

Teniendo en cuenta lo anterior, es deseo de la solicitante manifestar respetuosamente su desacuerdo y presentar argumentos en contra de estas declaraciones, con el fin de demostrar que la invención reclamada en la presente solicitud posee características técnicas que implicaron un paso inventivo durante su desarrollo.

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Respecto a lo indicado por el señor examinador en la Tabla de "Características esenciales" del concepto técnico que se contesta, la solicitante desea manifestar que contiene varios errores, entre los que se encuentran:

- Una "composición de ciclodextrina" no es una película o una lámina. Los elementos de D1 y D2 citados como "composición de ciclodextrina" son películas o láminas de material. La Reivindicación 1 es una composición que comprende monómeros polimerizables con radiación - **no polímeros**. Los monómeros son típicamente líquidos y en cualquier caso no pueden realizarse en una película cohesiva o una lámina.
- "Uno o más monómeros polimerizables con radiación" (o la modificación y el estrecho grupo de monómeros) no es un polímero. D1 no revela monómeros, sólo polímeros. Por lo cual, la referencia del señor examinador de D1 es un error.
- "Un complejo de inclusión ciclodextrínico comprende (...) un inhibidor olefínico (...)" no es revelado por D2. Ni ciclodextrina ni un inhibidor olefínico se describen en D2. El señor examinador parece señalar que uno de la ciclodextrina, el inhibidor olefínico, o el complejo del mismo es una composición antibacteriana. Por lo cual, esto es un error.

Además, el señor examinador afirma que la única diferencia entre D1 y la invención reclamada es que D1 no revela monómeros polimerizables por radiación y, D2 divulga monómeros polimerizables por radiación, por lo cual, las reivindicaciones presentadas carecen de actividad inventiva. Además, el señor examinador afirma que no hay ningún efecto técnico inesperado de la composición reclamada y que no hay estudios de estabilidad de almacenamiento, por ejemplo de productos frescos, que estén dentro de la descripción.

Con respecto a la falta de estudios de estabilidad de almacenamiento, la solicitante desea manifestar respetuosamente que, los efectos del 1-metilciclopropeno (1-MCP) y compuestos relacionados con ciclopropeno son bien conocidos como se puede apreciar en el documento **Sisler, E. and Serek, M., "Compounds controlling the ethylene receptor", Bot. Bull. Acad. Sin. (1999) 40: 1-7**, el cual se presenta como anexo a este memorial.

Dado que 1-MCP es un gas, la solicitante midió el espacio superior que rodea las composiciones de la presente invención para determinar la presencia de gas libre de 1-MCP liberado por las composiciones reclamadas. Una vez presente en la fase gaseosa, los efectos de la molécula de 1-MCP no son diferentes de los efectos del gas liberado

por algún otro método. La invención reclamada está dentro del conjunto de las composiciones reivindicadas, y no en la novedad o efectos de la molécula de 1-MCP.

Con respecto al efecto técnico inesperado de la composición, la solicitante desea presentar la siguiente información con respecto a D1, D2, y D1 en combinación con el documento EP 1593306.

En primer lugar, es importante entender los problemas de estabilidad que rodean el complejo 1-MCP/c/CD, que es una composición divulgada primero por **Daly en el documento US 6,017,849** (el cual se adjunta al presente memorial). Esta información es útil para mostrar cómo la composición reclamada suministra un efecto técnico inesperado y también resuelve un problema que no puede ser solucionado por cualquier combinación de las referencias citadas.

En resumen, el complejo es inestable en presencia de calor y solventes y se descompone para formar 1-MCP y ciclodextrina. Los ciclopropanos tales como 1-MCP son gases a temperaturas ambiente comunes; el 1-MCP tiene un punto de ebullición de 4.7°C. Por lo tanto, una vez que el complejo se descompone, el gas se evapora y el rendimiento de 1-MCP/c/CD se pierde.

El complejo 1-MCP/c/CD se descompone en presencia de solventes. Hotchkiss, et al., *Food Sci.* 2007 Jun; 72 (5): E330-4 (el cual se adjunta al presente memorial), estudiaron la liberación de 1-MCP a partir de películas de polímero después de la disolución de resinas de polímero en disolventes junto con 1-MCP/c/CD, seguido por fundición y secado de la combinación a temperatura ambiente para formar películas (Hotchkiss, E331).

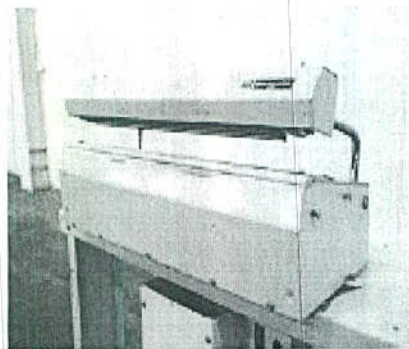
Los investigadores encontraron que "sólo trazas de 1-MCP" fueron liberados posteriormente de la película fundida (Id., E332). De este modo, el complejo de 1-MCP/c/CD libera el 1-MCP cuando entra en contacto con disolventes.

El complejo de 1-MCP/c/CD se descompone cuando se calienta por encima de 90°C. El análisis detallado del complejo 1-MCP/c/CD mediante calorimetría diferencial de barrido muestra que el inicio de la liberación térmicamente disparada de 1-MCP se produce a 90°C (Neoh, et al., *J. Phys. Chem. B* 2008, 112, 15914-15920, en Resumen y Figura 2, el cual se adjunta al presente memorial). Además, un estudio de 1-MCP liberado por películas de polímero después de la compresión en estado fundido con 1-MCP/c/CD se llevó a cabo por Hotchkiss et al. (mencionado anteriormente), en el que el moldeo durante 1 minuto a temperaturas de 115°C, 135°C, y 142°C resultó en pérdida significativa y creciente de 1-MCP; las temperaturas de moldeo de 165°C y superiores durante 1 minuto resultaron en la pérdida completa esencialmente de 1-MCP.

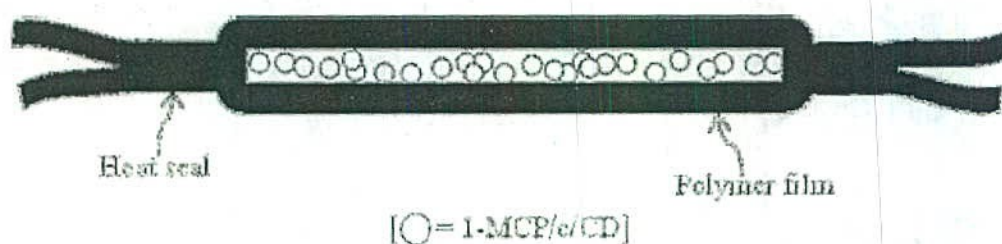
Ahora bien, D1 describe 1-MCP/c/CD "incorporado" en los materiales de empaque. Sin embargo, no hay ninguna descripción de cómo se lleva a cabo la incorporación. El párrafo [0017] afirma que *"ciclopropanos encapsulados se pueden mezclar dentro, o laminado entre, diferentes plásticos de empaque termoplástico... incorporados en varias ceras, papeles recubiertos, y cartón o puede ser en incorporado en un componente adhesivo de materiales de embalaje o incorporado en las etiquetas del paquete"*. No hay descripciones generales adicionales con respecto a cómo se podría llevar a cabo la incorporación de los ciclopropanos encapsulados.

En el caso de 1-MCP/c/CD, no es suficiente para indicar un interés por incorporar el complejo en una matriz polimérica. Más bien, es necesario explicar cómo la incorporación puede llevarse a cabo. Debido al caso especial de 1-MCP/c/CD, que es termolíticamente y solvolíticamente inestable.

Los Ejemplos 16 a 18 de D1 (Párrafos [0097] - [0099]), pretenden incorporar 1-MCP/c/CD en un material polimérico. En los ejemplos 16 y 17 el 1-MCP/c/CD en polvo, se sella al calor en una película de espesor de 1,5 mm de alcohol de polivinilo o 2,0 mm de espesor de una película de polietileno de baja densidad, respectivamente. El aparato de sellado por calor, un sellador de calor Sealmaster 420, tiene un único, elemento lineal de calor hacia el borde de sellado de dos capas de película juntas (similar a un aparato de sellado de comida casera). Imágenes de la Sealmaster 420, utilizada en los Ejemplos, se presentan a continuación:



Por lo tanto, la incorporación de 1-MCP/c/CD en un material polimérico, en los Ejemplos 16 y 17 de D1, implica la colocación del 1-MCP/c/CD en polvo entre dos capas de película (como plegado de la película o el uso de dos secciones de película) y sellar por calor los bordes para formar una "bolsa" que encierra el complejo CD:



Ejemplo 18 emplea una película de parafina (Parafilm® M) disuelta en hexano y mezclada con el complejo CD, seguido por secado de la mezcla a condiciones ambiente. Primero, es importante reconocer que una parafina no es un polímero, tal como una poliolefina. Más bien, las parafinas son ceras que tienen la fórmula química $C_nH_{(2n+2)}$, en el que $n = 24$ a 36 .

NOTA: En cuanto a las parafinas como especies no poliméricas, véase, por ejemplo http://www.chemicalbook.com/ChemicalProductProperty_EN_CB2854418.htm.

Alternativamente, una referencia que es anterior a la presente solicitud es **B. Musser y P. Kilpatrick, *Energy & Fuels* 1998, 12, 715-725** (el cual se adjunta al presente memorial) el cual divulga que las ceras de parafina tienen pesos moleculares en el rango de 350-600 g/mol (Resumen) ; y proporcionando además, una distribución de peso molecular ideal para una cera de parafina (Page 722 en la Figura 7, en referencia a la cera Malu Isan, o MI, caracterizada como una parafina en la Página 719 bajo "Análisis Elemental") y una "estructura promedio" de una cera de parafina (MI, Página 724 en la Figura 12).

En segundo lugar, al poner en contacto el 1-MCP/c/CD con un disolvente resultará en una pérdida sustancial de 1-MCP de 1-MCP/c/CD, según lo establecido en la discusión de la referencia Hotchkiss arriba mencionada. La Tabla 5 de D1 proporciona evidencia de pérdida de 1-MCP debido a la fusión con disolvente. La cantidad de 1-MCP liberado en el Ejemplo 18, como se muestra en la Tabla 5, es muy bajo en comparación con la cantidad liberada de la bolsa de poliolefina del Ejemplo 17 (Tabla 4). Esto es cierto, a pesar de que las ceras son similares en términos de hidrofobicidad y otras propiedades de las poliolefinas por lo que un experto podría esperar para afectar la velocidad de liberación. Los bajos niveles de 1-MCP liberados de la cera son más probablemente el resultado de una pérdida significativa de 1-MCP antes de la prueba.

Los tres ejemplos descritos anteriormente, más la simple divulgación en la descripción citada anteriormente, son las únicas referencias en D1 que proporcionan información acerca de cómo un experto podría incorporar el 1-MCP/c/CD en un material de envasado. El 1-MCP /c/CD **No se incorporó** en realidad en una matriz de polímero, y

no hay divulgación alguna, informando a una persona versada en la materia de cómo incorporar el 1-MCP/c/CD en una matriz de polímero como se encuentra en D1.

La falla de D1 para permitir en realidad cualquier incorporación de 1-MCP/c/CD en una matriz polimérica, o incluso sugerir cómo se podría hacer una cosa así, es significativa. Como se discute en detalle más arriba, el 1-MCP/c/CD es termolíticamente y solvolíticamente inestable. Por lo tanto, el problema de cómo podría ser incorporado el 1-MCP/c/CD en una matriz polimérica, es decir, dentro de una película de polímero a diferencia de sellado dentro de una bolsa construida de una película de polímero, no es trivial.

No es suficiente para afirmar que "combinando" (lo que un experto en la materia entiende en el sentido de mezclar en estado fundido o mezcla con disolvente) se podría utilizar para lograr la incorporación. En el caso especial de 1-MCP/c/CD, es necesario presentar cómo se podría incorporar el 1-MCP/c/CD en un recubrimiento o película sin pérdida significativa o total de 1-MCP. Como se evidencia, D1 no muestra esto.

Por otro lado, D2 describe un recubrimiento curable por radiación que tiene un (unos) compuesto(s) antimicrobiano(s) que está recubierto sobre un sustrato y se cura por irradiación (Columna 1, líneas 28-32). D2 no describe ningún uso de ciclodextrina, o 1-MCP, o 1-MCP/c/CD, o cualquier otro complejo de ciclodextrina. La solicitante llama la atención al señor examinador en que el 1-MCP, CD y 1-MCP/c/CD no son materiales antibacterianos.

Con respecto al documento EP 1593306, dicho documento describe combinaciones de 1-MCP/c/CD con materiales higroscópicos o delicuescentes, incluyendo poliéteres tales como polietilenglicol. EP 1593306 describe, además, el revestimiento de las combinaciones sobre un sustrato. Los ejemplos incluyen la fundición de disolvente de acetona (Ejemplo 2, párrafo [0022]) y la combinación de 1-MCP/c/CD con polietilenglicol y recubrimiento de la combinación sobre un sustrato utilizando técnicas no reveladas (Ejemplos 3-6, párrafos [0023] a [0026]). En los ejemplos citados de EP 1593306, la liberación posterior de 1-MCP fue completa dentro de 2 horas (Ejemplos 3-6) a 4 horas (Ejemplo 2).

Ahora bien, los problemas de estabilidad que rodean al 1-MCP/c/CD son discutidos anteriormente en detalle. Puesto que, es conocido que el contacto del complejo 1-MCP/c/CD con disolventes o la exposición del complejo a temperaturas por encima de 90°C causará una pérdida significativa de 1-MCP a partir del complejo, fue sorprendente para la solicitante que el 1-MCP/c/CD fue determinado como estable en presencia de monómeros curables con radiación en fase líquida tales como ésteres de ácido acrílico y de acrilato. Los monómeros que son líquidos a

temperaturas ambiente de laboratorio pueden ser considerados como disolventes polimerizables. Dichos monómeros incluyen especies empleadas comúnmente, tales como ácido acrílico y acrilato de butilo. Debido a que el trabajo de *Hotchkiss et al.* mostró la inestabilidad solvolítica del 1-MCP/c/CD, la solicitante no esperaba que el complejo de 1-MCP/c/CD fuera estable cuando se adiciona a tales líquidos.

Los Ejemplos 3-5 de la presente invención implican la adición de 1-MCP/c/CD a una mezcla curable de rayos UV de un proveedor (Vegra capa ULTRAVIOLETA VP 10169/60 MF-2NE). El Ejemplo 6 implica la adición de 1-MCP/c/CD con una composición que incluye ácido acrílico y acrilato de isooctilo. No se reportó la pérdida de 1-MCP.

Aunque la referencia D2 describe un recubrimiento curable por radiación, no podía ser previsto por el experto en la materia antes del descubrimiento de la solicitante de que 1-MCP/c/CD sería estable en presencia del recubrimiento líquido curable por radiación. D1 no está dirigido a monómeros curables por radiación o curado por radiación.

Con base en el análisis anterior, ni D1 ni D2 ya sea solos o en combinación, enseñan o sugieren la presente invención. En vista de lo anterior, **las nuevas reivindicaciones presentadas no son obvias y por lo tanto son inventivas sobre el estado del arte.** Por lo tanto, se le solicita respetuosamente al examinador retire la objeción de nivel inventivo.

Respecto a los otros grupos inventivos señalados por el señor examinador en el concepto técnico que se contesta, la solicitante desea manifestar respetuosamente que, las composiciones curadas de la nueva reivindicación 23 se forman sometiendo las composiciones de las nuevas reivindicaciones 1 a 6 a los métodos de las nuevas reivindicaciones 20 a 22. Las composiciones curadas se pueden formar en un material de envasado (nuevas reivindicaciones 7 a 12); el material de envasado así formado se emplea de forma útil en un recipiente (nuevas reivindicaciones 13 a 19).

En cuanto a las ventajas técnicas del método reclamado en las nuevas reivindicaciones 20 a 22, con el conocimiento de la solicitante, no ha habido estudios previos de la estabilidad de 1-MCP/c/CD cuando se somete a la radiación UV. A la luz de la discusión anterior con respecto a las inestabilidades asociadas con 1-MCP/c/CD, no se podría decir que la estabilidad del 1-MCP/c/CD cuando se irradia con radiación UV fue esperada antes a la divulgación de la presente invención.

Esta estabilidad se transformó en una técnica útil, según las reivindicaciones 20 a 22, para formar una matriz polimérica que tiene 1-MCP/c/CD arrastrado en el mismo. Debido a las inestabilidades termolíticas y solvolíticas asociados con el 1-MCP/c/CD,

los métodos de formación de polímeros que tienen el complejo mezclado en el mismo no están dentro del ámbito de un experto versado en la materia para formar mezclas de polímeros. Por lo tanto, ni D1 ni D2 describen ningún método de recubrimiento empleando 1-MCP/c/CD.

EP 1593306 describe un solvente fundido de 1-MCP/c/CD mezclado con acetona. El disolvente fundido conduce a al menos una cierta pérdida de 1-MCP, como se demostró por *Hotchkiss*. EP 1593306 describe otros recubrimientos en el que el método de hacer el recubrimiento no se da a conocer. Sin más detalles sobre cómo se realizaron estos recubrimientos, un experto entendería que el recubrimiento de disolvente o el recubrimiento fundido fueron desarrollados, dado que, estas son las dos técnicas comúnmente empleadas para formar recubrimientos poliméricos.

Dado que ambas técnicas de recubrimiento demuestran que resulta al menos una cierta pérdida de 1-MCP/c/CD, la composición curada de ciclodextrina de la presente invención tiene una ventaja técnica sobre EP 1593306 ya que, en la composición de las presentes reivindicaciones, no hay pérdida de 1-MCP generado por el procesamiento de las mezclas de monómeros para formar las composiciones curadas.

El solvente mediante mezclado y recubrimiento, similar al realizado por *Hotchkiss*, resulta en la descomposición de 1-MCP/c/CD. La mezcla y recubrimiento por medio de temperatura, tal como la mezcla de extrusión y similares, de manera similar conduce a la descomposición de 1-MCP/c/CD. El problema de cómo formar una matriz polimérica que tiene 1-MCP/c/CD mezclado en la misma no se resolvió antes de la presente invención.

Las ventajas técnicas del método incluyen la estabilidad del 1-MCP/c/CD durante el proceso; la facilidad de recubrimiento de un material que contiene 1-MCP/c/CD, dadas las inestabilidades asociadas con la capa de disolvente o el proceso de fundición; el uso de la metodología de curado UV convencional para lograr una composición curada que tiene 1-MCP/c/CD arrastrado en el mismo; y la capacidad para dirigirse hacia una amplia variedad de sustratos con facilidad. Las descripciones de las referencias citadas no permiten estas ventajas o proporcionan ninguna forma para un experto en cómo podría utilizar dichas características técnicas en un método.

Con respecto a la nueva reivindicación 23, como se presentó anteriormente, ni el contacto con monómeros curables por radiación ni la irradiación de la mezcla de monómeros con 1-MCP/c/CD resultan en la pérdida medible de 1-MCP del complejo.

La consecuencia de la combinación de estos dos resultados es una primera ventaja técnica: la Solicitante ha permitido la formación de una matriz polimérica que tiene un

complemento cuantitativo de 1-MCP/c/CD dentro de la matriz polimérica, basado en la cantidad adicionada a la composición de monómeros de las reivindicaciones 1 a 6 antes del recubrimiento y el curado utilizando los métodos de las reivindicaciones 20 a 22.

Ninguna de las referencias citadas enseña el método que permite la formación de un recubrimiento sin incurrir en pérdida de 1-MCP. Como se discutió anteriormente, D1 no enseña o habilita una manera de arrastrar 1-MCP/c/CD en una matriz polimérica, tal como dentro de un revestimiento. D2 no se dirige a 1-MCP/c/CD. Antes de la presente invención, un revestimiento polimérico o capa que tiene una cantidad conocida de 1-MCP/c/CD incorporado dentro del recubrimiento no se logró.

Una segunda ventaja técnica es el suministro de una composición que comprende 1-MCP/c/CD dentro de una matriz de polímero. Esta ventaja lleva inherentemente a la posibilidad de la formación de recubrimientos y capas que tienen 1-MCP/c/CD incorporados en las mismas:



Dado que no se mide la pérdida de 1-MCP del 1-MCP/c/CD añadido a la composición de monómero, y no es medible el 1-MCP que se pierde durante la transformación de la composición de monómero a la composición curada, una cantidad sustancialmente cuantitativa de 1-MCP/c/CD está presente en las composiciones curadas de la reivindicación 23 en base a la cantidad adicionada a la composición de monómero. Ninguna de las referencias citadas, permite tal composición.

Además, un usuario que forma una composición curada como la de la reivindicación 23 sabría exactamente cómo fue arrastrado el 1-MCP/c/CD en el recubrimiento polimérico. Esta es una ventaja técnica significativa, porque la cantidad de 1-MCP utilizado para inhibir la maduración o senescencia de productos frescos es generalmente inferior a 1 ppm en la atmósfera que rodea al producto.

Además, muchos países limitan la concentración permisible de 1-MCP gaseoso a ≤ 1 ppm. Aún más, el nivel óptimo de 1-MCP expuesto difiere para cada tipo de producto adecuado para el tratamiento con 1-MCP, dentro del ≤ 1 límite ppm. Con las estrictas regulaciones con respecto a la cantidad permitida de 1-MCP gaseoso y las variaciones en dentro del ≤ 1 ppm máximo, afecta el resultado del tratamiento. Esto es muy importante para un usuario, el saber exactamente cuánto de 1-MCP/c/CD está

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presente en cualquier composición utilizada para el propósito de prevenir la maduración o senescencia en productos frescos.

Sin embargo, otra ventaja técnica de las composiciones de la reivindicación 23 es la verdadera liberación controlada de 1-MCP de un recubrimiento, a partir de una composición curada. En primer lugar, como se señaló anteriormente, la cantidad de 1-MCP/c/CD que reside en las composiciones curadas tras la finalización del curado es conocido con certeza: es la cantidad adicionada a las composiciones de monómeros antes de la irradiación UV.

En segundo lugar, la liberación de 1-MCP se produce durante al menos 48 horas (Solicitud, Página 61, Tabla 4 y Página 68, Tabla 7); al menos 96 horas (Solicitud, Página 58, Tabla 1); o al menos 170 horas (Solicitud, Página 70, Tabla 8).

En tercer lugar, la velocidad de liberación con el tiempo es repetible y predecible. Las variaciones en la concentración de 1-MCP/c/CD en el recubrimiento (Solicitud, Figura 2 y Ejemplo 3; y Figura 4 y Ejemplo 5, y la variación en el área de la película recubierta seleccionada (Solicitud, Figura 3 y Ejemplo 4) resultan en cambios previsibles en la velocidad de liberación como se demuestra en los Ejemplos.

La liberación controlada de 1-MCP a partir de las composiciones curadas es directamente atribuible al hecho que el 1-MCP/c/CD es arrastrado dentro de una matriz de polímero curado. El agua debe difundirse en la matriz para llegar al complejo de CD y desencadenar la liberación de 1-MCP; el 1-MCP debe entonces difundirse fuera de la matriz para alcanzar el material de planta que está siendo tratado.

Además, la Solicitante señala que la selección cuidadosa de las composiciones de monómeros proporciona un control adicional de la velocidad de liberación en las composiciones curadas resultantes mediante el ajuste de la permeabilidad del polímero al agua, el 1-MCP, o ambos (Solicitud, Página 18). Ninguna de las referencias citadas proporciona esta ventaja técnica.

La liberación controlada permite el uso de 1-MCP en cantidades totales entregadas y la velocidad de entrega se ajusta fácil y confiable dentro de los parámetros de ajuste del gobierno. Además, puesto que la cantidad exacta de 1-MCP/c/CD se conoce por el usuario al principio, se selecciona fácilmente una cantidad objetivo de 1-MCP entregable a un volumen particular o para un tipo particular de producto, por ejemplo, cortando una película que tiene la composición curada sobre el mismo se proporciona una cantidad específica de 1-MCP basado en una base de peso o volumen.

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Finalmente, con respecto a las reivindicaciones 7 a 12 y 13 a 19, el señor examinador clasificó estos dos grupos de reivindicaciones en grupos inventivos separados, de hecho se relacionan en que las reivindicaciones 7 a 12 se dirigen a un sustrato sobre el que se recubre una composición de la reivindicación 23; y en las reivindicaciones 13 a 19 se dirigen a un recipiente que emplea un sustrato recubierto con una composición de la reivindicación 23. Como tal, estas combinaciones de sustratos y composiciones de polímeros curados comparten el elemento común de una composición polimérica curada (como se reivindica en la reivindicación 23). Las ventajas técnicas descritas anteriormente para las composiciones de la reivindicación 23 se aplican con igual fuerza a las composiciones dispuestas sobre un sustrato, en el que además el sustrato se emplea en un recipiente.

Otra ventaja técnica de los sustratos y recipientes de las reivindicaciones 7 a 19 es que pueden ser utilizados sin limitación en el envasado de productos utilizando cualquiera de la gran cantidad de métodos utilizados para envasar productos y en cualquiera de la gran variedad de formatos empleados como "materiales de embalaje" o "contenedores". Todos los formatos y métodos resultan en una verdadera liberación controlada de 1-MCP.

En contraste, D1 permite sólo bolsas que sostienen 1-MCP/c/CD en polvo. D1 no divulga recubrimientos. Una bolsa se no se puede cortar aparte para dividir los contenidos sin botarlos, mientras que los materiales de empaque de las reivindicaciones 7-12, por ejemplo, se cortan fácilmente o de otro modo se dividen para seleccionar una cantidad de 1-MCP/c/CD para un uso seleccionado. Ahora bien, EP 1593306 describe combinaciones de 1-MCP con polímeros que reportan rápidamente pérdidas de 1-MCP. EP 1593306 no describe una composición de liberación controlada.

En este orden de ideas, es claro que el análisis de nivel inventivo realizado por el señor examinador es el resultado de una evaluación del tipo "*hindsight*" o retrospectivo. De hecho, este tipo de análisis no es permitido para desvirtuar el concepto de altura inventiva.

Por consiguiente, teniendo en cuenta los argumentos presentados, se concluye que las enseñanzas de las anterioridades citadas por su Despacho no llevan a un técnico versado en la materia a obtener la presente invención ya que no revelan o enseñan las características técnicas esenciales relacionadas con la misma, tal como acá es reivindicada. Lo anterior si se tiene en cuenta que para el estudio de nivel inventivo el Examinador no puede adoptar la posición de un inventor y que además no puede partir de la solución dada sino simplemente de lo que expresamente revelen el o los documentos más cercanos del estado del arte.

En este tipo de análisis, el señor examinador no enfoca su análisis en el momento en el que el solicitante plantea un problema con el fin de solucionar fallas técnicas existentes en los productos convencionales de un campo técnico específico, sino que se enfoca en la solución dada al problema planteado y en la búsqueda de documentos con conceptos análogos. A este respecto dirijo la atención del examinador al “Manual para Examen de Solicitudes de Patentes de Invención en las Oficinas de Propiedad Industrial de los Países de la Comunidad Andina, publicado en la página Web de la Comunidad Andina, donde se establece en la página 74 en cuanto al nivel inventivo “...que la búsqueda de anterioridades se efectúa a posteriori, tomando como punto de partida la misma invención,...el examinador debe realizar el esfuerzo intelectual de colocarse en la situación que ha tenido que afrontar el técnico con conocimientos medios en la materia en un momento en que la invención no era conocida, es decir antes de la invención”. (El subrayado es nuestro).

En el presente caso, para el señor examinador puede resultar “**obvio**” que el objeto de la presente solicitud se derive de la materia objeto de los documentos citados, sin dar mayor importancia al trabajo desplegado y a la capacidad inventiva del solicitante para lograr llegar a la solución del problema planteado. Es decir, que el señor examinador no hace su análisis partiendo del problema mismo que existía en la técnica en el momento en que la solicitante dirige todos sus esfuerzos para encontrar una solución, sino que enfoca su estudio en el momento en que el problema ya fue resuelto.

En cuanto a las características técnicas antes mencionadas relacionadas con la nueva reivindicación 1 de la presente invención, nos permitimos hacer hincapié en lo argumentado en la interpretación prejudicial 81-IP-2006 del Tribunal que ha establecido lo siguiente: “Ante ello, es preciso definir lo que se entiende por “obvio” y por “evidente”. Lo obvio es aquello que salta a la vista de manera clara y directa; lo que se encuentra o pone delante de los ojos o que no tiene dificultad. Mientras que lo evidente es algo tan cierto y claro que nadie puede dudar racionalmente de ello; se presenta de manera tan perceptible y manifiesta que muestra certeza y claridad¹. Como se podrá ver, la diferencia radica en lo “tan”, de forma que algo que resulte obvio no es necesariamente evidente; empero lo que es evidente, es también obvio.” (El subrayado es nuestro).

Es claro que la presente invención **no salta a la vista** de una persona medianamente versada en la técnica cuando se cuenta con la información de D1, D2 y EP 1593306.

¹ Diccionario de la Lengua Española. Vigésima Primera Edición. Madrid – España. 1992. Definiciones de evidente, evidencia y obvio. Pp. 655 y 1036.

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En este sentido, y siguiendo el Manual Andino de Patentes, la presente invención tal y como se encuentra definida en las nuevas reivindicaciones 1 a 23, tiene diferencias con el estado del arte e implicó un nivel inventivo durante su desarrollo, por lo cual debe ser considerada como novedosa e inventiva según las disposiciones de los artículos 16 y 18 de la Decisión 486.

5. Conclusiones

Habiendo atendido cabalmente las objeciones presentadas por ese despacho, atentamente solicitamos se conceda el privilegio de patente de invención a la solicitud en estudio o si el señor examinador considera que subsisten razones para objetar la presente invención, rogamos expedir otro examen de fondo con los debidos motivos y razones que sustenten su objeción con el fin de que nuestro mandante tenga la posibilidad de exponer sus argumentos con base en la interpretación de los artículos 45 de la Decisión 486 -el cual otorga la facultad al examinador para emitir los exámenes de fondo-, 35 y 59 del Código Contencioso Administrativo que señala que: *"habiéndose dado oportunidad a los interesados para expresar sus opiniones, y con base en las pruebas e informes disponibles, se tomará la decisión que será motivada al menos en forma sumaria si afecta a particulares"* y *"(..) deberá proferirse la decisión definitiva. Esta se motivará en sus aspectos de hecho y de derecho, y en los de conveniencia si es el caso"*, respectivamente (El subrayado es nuestro).

En consecuencia, solicitamos a ese despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional

Señor Superintendente,


CAROLINA DAZA MONTALVO
C.C. 66.783.790 de Palmira Valle
TP-141563 CSJ
notificaciones@clarkemodet.com.co

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

E. S. D.

Re: Expediente No. 13-252.771

Solicitud de patente a nombre de CELLRESIN TECHNOLOGIES, LLC

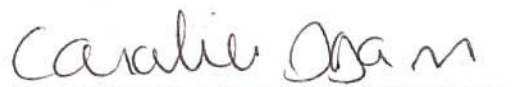
RESPUESTA ACCIÓN OFICIAL

Yo, SILVANA MARÍA LÓPEZ DÍAZ, identificada como aparece al pie de mi firma, domiciliada en Bogotá, atentamente informo a usted que sustituyo el poder con que obro, a la doctora CAROLINA DAZA MONTALVO, domiciliada en Bogotá, identificada con la Cédula de Ciudadanía No.66.783.790 de Palmira Valle y portadora de la tarjeta profesional No.TP-141563 del CSJ, con las mismas facultades que me fueron conferidas para este caso.

Muy atentamente,


SILVANA MARÍA LOPEZ DÍAZ
C.C. No. 22.468.787
TP- 147463 CSJ

Acepto sustitución


CAROLINA DAZA MONTALVO
C.C. 66.783.790 de Palmira Valle
TP-141563 CSJ

PRESENTACION PERSONAL

NOTARIA
11

El anterior escrito fue presentado ante el Notario 11 del Círculo de Bogotá personalmente por SILVANA MARIA LOPEZ DIAZ quien exhibió CC No 22.468.787 de BARRANQUILLA y Tarjeta Profesional de Abogado No.147463 del C.S.J.C y declaró que el contenido del mismo es cierto. Bogotá D.C. 30/04/2015



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PRESENTACION PERSONAL

NOTARIA
11

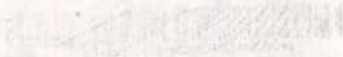
El anterior escrito fue presentado ante el Notario 11 del Círculo de Bogotá personalmente por SILVANA MARIA LOPEZ DIAZ quien exhibió CC No 22.468.787 de BARRANQUILLA y Tarjeta Profesional de Abogado No.147463 del C.S.J.C y declaró que el contenido del mismo es cierto. Bogotá D.C. 30/04/2015



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11

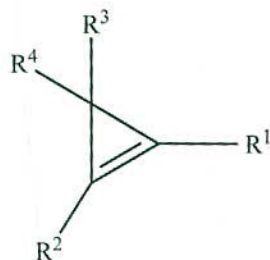
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ANEXO

- Nuevo capítulo de reivindicaciones
- Sisler, E. and Serek, M., "*Compounds controlling the ethylene receptor*"
- US 6,017,849
- *El complejo 1-MCP/c/CD se descompone en presencia de solventes.*
Hotchkiss, et al.
- Neoh, et al., *J. Phys. Chem*
- B. Musser y P. Kilpatrick, *Energy & Fuels*

REIVINDICACIONES

1. Una composición de ciclodextrina que comprende uno o más monómeros polimerizables con radiación, el o los monómeros polimerizables con radiación comprenden ácido acrílico, ácido metacrílico, un éster acrilato, un éster metacrilato, una acrilamida, un diacrilato, un triacrilato, un tetraacrilato o una mezcla de estos y entre un 0.001%p y un 25%p de un complejo de inclusión ciclodextrínico en función del peso de la composición, comprendiendo el complejo de inclusión ciclodextrínico una composición de ciclodextrina y un inhibidor olefínico de la generación de etileno en un producto, comprendiendo el inhibidor olefínico un compuesto que tiene la estructura



donde cada uno de los grupos R^1 y R^2 es independientemente hidrógeno o un grupo hidrocarbilo C_{1-16} , y R^3 y R^4 son independientemente hidrógeno o un grupo hidrocarbilo C_{1-16} con la condición de que al menos uno de los grupos R^1 y R^2 sea metilo.

2. La composición de la reivindicación 1 donde la composición comprende además un fotoiniciador.

3. La composición de la reivindicación 1 donde la composición comprende además uno o más prepolímeros.

4. La composición de la reivindicación 1 donde el inhibidor olefínico comprende 1-metilciclopropeno.

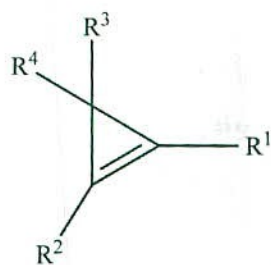
5. La composición de la reivindicación 4 donde la composición de ciclodextrina comprende α -ciclodextrina.

6. La composición de la reivindicación 1 donde la composición comprende entre un 0.01%p y un 10%p del complejo de inclusión ciclodextrínico en función del peso de la composición.

7. Un material de envasado tratado que comprende un material de envasado y una composición de ciclodextrina curada dispuesta sobre al menos una porción de una superficie del material de envasado, comprendiendo esencialmente la composición de ciclodextrina curada

un polímero derivado de uno o más monómeros polimerizables con radiación seleccionados del grupo constituido por ácido acrílico, ésteres alquílicos del ácido acrílico, ésteres alquílicos del ácido metacrílico, monómeros con más de un grupo polimerizable insaturado y mezclas de estos;

entre un 0.001%p y un 25%p de un complejo de inclusión ciclodextrínico en función del peso de la composición, comprendiendo el complejo de inclusión ciclodextrínico ciclodextrina y un inhibidor olefínico de la producción de etileno en productos, comprendiendo el inhibidor olefínico un compuesto que tiene la estructura



donde cada uno de los grupos R^1 y R^2 es independientemente hidrógeno o un grupo hidrocarbilo C_{1-16} , y R^3 y R^4 son independientemente hidrógeno o un grupo hidrocarbilo C_{1-16} con la condición de que al menos uno de los grupos R^1 y R^2 sea metilo.

8. El material de envasado tratado de la reivindicación 7 donde el material de envasado tratado comprende una película, una lámina, una bolsa, una cesta, una bandeja, un bote, una tapa, una etiqueta, un cartoncillo o una inserción para envases tratada.

9. El material de envasado tratado de la reivindicación 7 donde el material de envasado comprende una poliolefina o un poliéster.

10. El material de envasado tratado de la reivindicación 7 donde la composición de ciclodextrina curada comprende un adhesivo piezosensible.

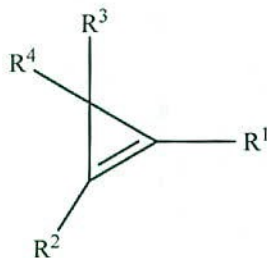
11. El material de envasado tratado de la reivindicación 7 donde la composición de ciclodextrina curada comprende marcas impresas o en relieve.

12. El material de envasado tratado de la reivindicación 7 donde el material de envasado tratado es un laminado tratado.

13. Un recipiente que comprende un material de envasado tratado, donde el recipiente comprende un volumen cerrado, comprendiendo el material de envasado tratado una composición de ciclodextrina curada dispuesta sobre al menos una porción de una superficie del material de envasado, comprendiendo esencialmente la composición de ciclodextrina curada

un polímero derivado de uno o más monómeros polimerizables con radiación seleccionados del grupo constituido por ácido acrílico, ésteres alquílicos del ácido acrílico, ésteres alquílicos del ácido metacrílico, monómeros con más de un grupo polimerizable insaturado y mezclas de estos;

entre un 0.001%p y un 25%p de un complejo de inclusión ciclodextrínico en función del peso de la composición, comprendiendo el complejo de inclusión ciclodextrínico ciclodextrina y un inhibidor olefínico de la producción de etileno en productos, comprendiendo el inhibidor olefínico un compuesto que tiene la estructura



donde cada uno de los grupos R¹ y R² es independientemente hidrógeno o un grupo hidrocarbilo C₁₋₁₆, y R³ y R⁴ son independientemente hidrógeno o un grupo hidrocarbilo C₁₋₁₆ con la condición de que al menos uno de los grupos R¹ y R² sea metilo.

14. El recipiente de la reivindicación 13 donde el recipiente es un recipiente laminado tratado.

15. El recipiente de la reivindicación 13 donde la composición de ciclodextrina curada está presente como un revestimiento sobre una inserción para envases.

16. El recipiente de la reivindicación 13 que comprende un artículo de producto.

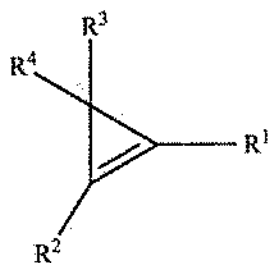
17. El recipiente de la reivindicación 13 donde el volumen cerrado comprende una humedad relativa de entre el 50% y el 100% a una temperatura de entre 0 °C y 20 °C.

18. El recipiente de la reivindicación 13 donde el volumen cerrado comprende una humedad relativa del 100% a una temperatura de entre 0 °C y 20 °C, y comprende además agua líquida.

19. El recipiente de la reivindicación donde 13 el inhibidor olefínico está presente en el volumen cerrado en una concentración comprendida entre 2.5 partes por billón y 10 partes por millón.

20. Un método para preparar un material de envasado tratado, comprendiendo el método

a. formar una composición de ciclodextrina que comprende uno o más monómeros polimerizables con radiación, el o los monómeros polimerizables con radiación comprenden ácido acrílico, ácido metacrílico, un éster acrilato, un éster metacrilato, una acrilamida, un diacrilato, un triacrilato, un tetraacrilato o una mezcla de estos y entre un 0.001%p y un 25%p de un complejo de inclusión ciclodextrínico en función del peso de la composición de ciclodextrina, comprendiendo el complejo de inclusión ciclodextrínico ciclodextrina y un inhibidor olefínico de la generación de etileno en productos, comprendiendo el inhibidor olefínico un compuesto que tiene la estructura



donde cada uno de los grupos R^1 y R^2 es independientemente hidrógeno o un grupo hidrocarbilo C_{1-16} , y R^3 y R^4 son independientemente hidrógeno o un grupo hidrocarbilo C_{1-16} con la condición de que al menos uno de los grupos R^1 y R^2 sea metilo;

b. disponer la composición de ciclodextrina sobre al menos una porción de una superficie de un material de envasado con un grosor de 0.01 micrones a 1 milímetro para formar un revestimiento; y

c. exponer el revestimiento a una fuente de radiación para formar una composición de ciclodextrina curada.

21. El método de la reivindicación 20 donde la composición de ciclodextrina comprende además entre un 0.1%p y un 5%p de uno o más fotoiniciadores en función del peso de la composición, donde la fuente de radiación es radiación ultravioleta.

22. El método de la reivindicación 20 donde la composición de ciclodextrina comprende además entre un 0.1%p y un 5%p de uno o más fotoiniciadores en función del peso de la composición; y que comprende además exponer la composición de ciclodextrina a radiación ultravioleta antes de la disposición.

23. Una composición de ciclodextrina curada, comprendiendo esencialmente la composición:

un polímero derivado de uno o más monómeros polimerizables con radiación seleccionados del grupo constituido por ácido acrílico, ácido metacrílico y ésteres alquílicos del ácido acrílico o metacrílico, donde los grupos éster tienen entre 1 y 18 carbonos, monómeros con más de un grupo polimerizable insaturado y mezclas de estos;

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Mayo 6, 2015

entre un 0.001%p y un 25%p de un complejo de inclusión ciclodextrínico en función del peso de la composición, comprendiendo el complejo de inclusión ciclodextrínico ciclodextrina y 1-metilciclopropeno.

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Bot. Bull. Acad. Sin. (1999) **40**: 1_7

Sisler and Serek — Compounds controlling the ethylene receptor

(Invited review paper)

Compounds controlling the ethylene receptor

Edward C. Sisler^{1,3} and Margrethe Serek²

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Abstract. Many organic compounds interact with the ethylene receptor and diffuse free after different periods of time. Some are agonists and mimic ethylene, and some are antagonists, which prevent ethylene action by blocking the receptor. Some of the antagonists have proven useful in scientific studies, and some promise to be commercially important in protecting against ethylene. The times that different compounds remain bound may be important clues to how ethylene transmits its signal to the signal transduction pathway. Ethylene diffuses from the site with a $t_{1/2}$ of 2_10 minutes and is an active compound. For 2,5-Norbornadiene (2,5-NBD) and some other strained olefins that block ethylene action, the half diffusion time is 3_6 h. For other more strained compounds (cyclopropenes) the half diffusion time is estimated to be 7_12 days; they block ethylene action during this time. The time of diffusion from the receptor appears to be the major difference between compounds that block the receptor and those that are active. It is suggested that this time constant may be the controlling factor in ethylene action.

Keywords: 1-methylcyclopropene, 1-MCP; 2,5-norbornadiene, 2,5-NBD; 3,3-dimethylcyclopropene, 3,3-DMCP; Cyclopropene; Diazocyclopentadiene, DACP; Ethylene antagonist; Ethylene receptor; Methylenecyclopropane; *trans*-Cyclooctene.

Abbreviations: 1-MCP, 1-methylcyclopropene; 2,5-NBD, 2,5-norbornadiene; 3,3-DMCP, 3,3-dimethylcyclopropene; DACP, diazocyclopentadiene.

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Introduction

In recent years some very effective agents for blocking the ethylene receptor have been discovered by Sisler and coworkers. These block ethylene action, and hold the promise of being a new way of controlling ripening, se

nescence, and other ethylene responses. Since the ethylene receptor is ubiquitous in plants, these compounds should control all ethylene responses in plants. Rather than being a totally new discovery, they are the consequence of efforts to develop a better understanding of the ethylene receptor. This paper is not intended to cover all of the many papers in which these compounds have been used, but instead to cover the important findings and uses of the compounds. We will initially address what has been

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learned from inhibitor studies in plants, and then address how these relate to the recent identification of a putative ethylene receptor from *Arabidopsis*. Some aspects of receptor blocking agents have recently been reviewed (Sisler and Serek, 1997).

Although ethylene has been recognized as a plant hormone since 1901 (Neljubov, 1901) and many

and Sisler, 1995). Some cyclopropenes block for shorter times and others appear to be inactive. These compounds appear not only to be of practical value as ethylene antagonists, but also may give valuable clues as to how ethylene is able to enter into signal transduction (Figure 1).

Uses of Commercial or Scientific Value

important discoveries of ethylene action have been made in the intervening years, the paper by Burg and Burg (1967), which proposed that the ethylene receptor acted *via* a metal, was the beginning of work at the receptor level. Burg and Burg (1967) reported that compounds such as ethylene, propylene, butene, and vinyl methyl ether bind to silver ion in the same order as their ability to induce ethylene responses in plants. They postulated that a metal was involved in ethylene action. To this date, no one has proved that the ethylene receptor contains a metal, but the evidence strongly supports that view.

Sisler and Pian (1973) reported that some cyclic olefins appeared to block ethylene responses rather than to induce a response. Of these, 2,5-norbornadiene (2,5-NBD) blocked at the lowest concentration, but all of these compounds require continuous exposure to be effective. Sisler and Yang (1984) showed that the relative blocking ability of these compounds also paralleled their ability to bind to silver ion and proposed ring strain as an important factor. It has never been definitively shown exactly how these compounds work although much more effective compounds have become available since that time.

A number of other compounds such as *cis*-butene, cyclopentene (Sisler and Yang, 1984), and some heterocycle compounds were subsequently shown to inhibit ethylene action. In 1990 it was shown that the highly strained *trans*-cyclooctenes, but not the less strained *cis*-cyclooctenes, were much more effective, in terms of concentration, than 2,5-NBD (Sisler et al., 1990). These also required continuous exposure to the tissues to be effective in blocking ethylene responses.

While searching for a photoaffinity

There are four compounds which are or have been used extensively in scientific investigations: 2,5-NBD, *trans*-cyclooctene, DACP and 1-methylcyclopropene (1-MCP).

1) 2,5-NBD (2,5 bicyclohepta-2,5-diene) is a liquid (bp 89 C) at room temperature and is sufficiently volatile to be applied in the vapor state either by allowing it to evaporate in the treating chamber or inserting it with a stream of air. Since it was the best of the first group of receptor blockers discovered, it has been used in numerous scientific studies to block the ethylene receptor. It has a very disagreeable odor but has the advantage of being commercially available. It is still being used in some studies. It must be continually applied to be effective, and at high

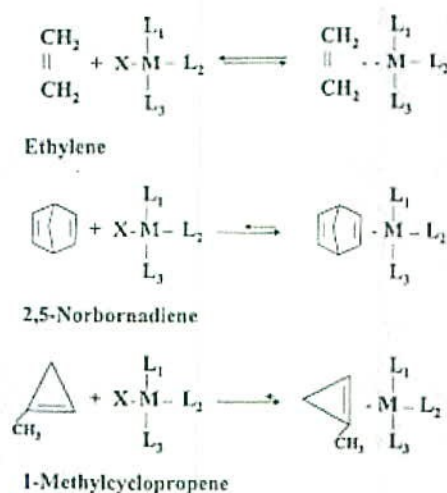


Figure 1. Types of interaction with

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label for the receptor (Sisler and Blankenship, 1993a,b), the irradiation product of diazocyclopentadiene (DACP) was shown to block the ethylene receptor for many days. The product appears to be a gas at room temperature; however, it is very unstable and has not been identified. The product of photolysis was shown to block ripening and senescence in banana (*Musa sapientum*), tomatoes (*Lycopersicon esculentum*), kiwi (*Actinidia chinensis*), persimmons (*Diospyros virginiana*), and carnation (*Dianthus caryophyllus*) and prevent ethylene inhibition of pea (*Pisum sativum*) growth (Sisler and Blankenship, 1993a,b; Sisler and Lallu, 1994; Serek et al., 1994). Using ^{14}C -ethylene, it has been shown to block the receptor in mung bean (*Vigna radiata*) sprouts and in tobacco (*Nicotiana tabacum*) leaves.

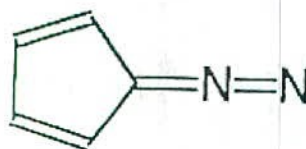
Subsequently, it was discovered that some cyclopropenes counteract the effects of ethylene for 10–12 days in tissues given a single exposure at very low concentrations (Sisler et al., 1996a,b; Serek et al., 1995; Dupille

the ethylene receptor. After binding, a rearrangement of the ligands in some unknown manner is thought to occur in each case. (Top) Ethylene and many other pi acceptor compounds bind to the receptor and induce an ethylene response. Ethylene, acetylene, carbon monoxide, and isocyanides are examples. (Middle) Strained olefin compounds which bind to the receptor and prevent ethylene responses but require continuous exposure. High levels of ethylene will overcome the effect by competition. 2,5 NBD and *trans*-cyclopropene are examples of these compounds. (Bottom) Strained olefins compounds which bind to the receptor and prevent ethylene responses for up to 12 days by a single exposure, during which time ethylene does not overcome the response. 1-MCP, cyclopropene and 3,3-DMCP are examples of these compounds.

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concentrations stimulate ethylene production which may overcome its effect if the ethylene is not vented away. The noxious odor limits its use to scientific investigations.

2) *trans*-cyclooctene is also a liquid (bp 75 °C, 100 torr) but is sufficiently volatile to be applied as a vapor and is a good inhibitor of ethylene responses. It is usually effective at concentrations 50–100 times lower than 2,5-NBD. This



diazocyclopentadiene

compound should not be confused with *cis*-cyclooctene, which is much less active and requires concentrations more than 600 times higher (Figure 2). Despite the superior properties of *trans*-cyclooctene, it has seen only limited use since it is not commercially available and must be synthesized (Hiyama and Nozaki, 1973) for use. It too must be continuously supplied to be effective and has an unpleasant odor.

3) DACP (bp 52_53, 50 torr) (Regitz and Leidhegener, 1967; Sisler and Blankenship 1993a,b) binds to the receptor but is not very effective in blocking ethylene action. In fluorescent light, it photodecomposes to form a very effective inhibitor of ethylene responses (Figure 3). The photolysis product has not been identified, and the use of DACP has been limited because it is not commercially available. It is potentially explosive when concentrated, and should be kept in a solution of pentane or other organic compound. Anyone using this compound should be aware of its explosive nature and avoid ground glass joints (which promote the decomposition of diazo compounds) and avoid shocking it. It can be crystallized from alcohol or pentane at -70°C .

Figure 3. Photolysis of DACP. Fluorescent light causes the photodisintegration of DACP. DACP interacts with the receptor and blocks it, but requires continuous exposure. The photolytic product blocks up to 12 days, but requires continuous exposure. After light activation the unidentified light product is concentration-wise 5000 times more active and blocks the receptor up to 12 days.

4) 1-MCP represents the best example of a group of active cyclopropene compounds based on concentration and stability considerations. This compound is being commercially developed by Biotechnologies for Horticulture Inc., 751 Thunderbolt Dr. Waltersboro, SC 29488, USA. It can be synthesized easily by those familiar with air sensitive reagents (Magid et al., 1970). This compound will probably be the ethylene inhibitor of choice for the immediate future and holds considerable commercial potential since at its active concentration of 0.5 nl.l^{-1} on carnation, it has no detectable odor and has not been reported to have toxic properties.

Effects of Concentration

A relatively high concentration of 2,5-NBD is required to block the receptor. Table 1 shows that 55,000 nl.l^{-1} are required to protect bananas. Like ethylene binding, 2,5-NBD binding is an equilibrium reaction, and if the 2,5-NBD is

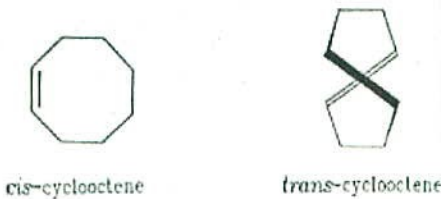


Figure 2. Structure of *cis*-cyclooctene and *trans*-cyclooctene. Both of these compounds block the ethylene receptor, but the highly strained *trans*-cyclooctene is concentration-wise 650 times more active than the *cis* form.

vented away, the plant material becomes sensitive to ethylene in a short time.

It requires 780 nl.l⁻¹ of *trans*-cyclooctene and continuous exposure to protect ethylene-induced ripening of bananas. 512,000 nl.l⁻¹ of *cis*-Cyclooctene is required for protection. Carnations exposed to DACP in the dark re

Table 1. Concentration of compound needed to protect plants against ethylene.

Compound Plant Concentration (nl.l ⁻¹)	
2-5-NBD Banana	55,000
<i>trans</i> -Cyclooctene Banana	780
<i>cis</i> -Cyclooctene Banana	512,000
DACP (dark) Carnation	700,000
DACP (light) Carnation	140
1-MCP Banana	0.7
1-MCP Carnation	0.5
1-MCP Pea growth	40
3,3-DMCP Banana	700

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quire 700,000 nl.l⁻¹ for protection against 10 µl.l⁻¹ of ethylene, but after the DACP is pre-irradiated with fluorescent light, 140 nl.l⁻¹ of DACP is required. This shows a considerable activation of effect. Some of this effect may be due to

appear that the difference between compounds that must be used continuously and those that are "permanent" is a matter of the dissociation time from the receptor (Figure 1).

How Long do Compounds Remain

the conversion to an unidentified product which binds more rapidly than DACP, but much of it probably is due to the fact that the unidentified product remains bound for many days while most of the unconverted DACP comes off the receptor within 60 minutes (Sisler et al., 1993).

To prevent ethylene induced ripening of bananas requires 2 nl.l^{-1} 1-MCP for a 6 h exposure or 0.7 nl.l^{-1} for a 24 h exposure. It is not known if this is an equilibrium reaction or not, but since the inhibition lasts for 12 days, in a practical sense it probably can be considered a non-equilibrium reaction. It is possible that at least part of the regained sensitivity to ethylene is due to the synthesis of new receptors. Although these values are for bananas, they can be considered approximate for most fruits and flowers.

Competition for the Receptor

Ethylene is the natural hormone for the receptor; however, other active compounds such as acetylene, carbon monoxide, isocyanides and other olefins compete for it (Burg and Burg, 1967; Sisler, 1977). Likewise, compounds that block the receptor compete with ethylene for it. 2,5-NBD (Sisler et al., 1985), *trans*-cyclooctene (Sisler et al., 1990) and many other cyclic olefins have competitive kinetics for the receptor. The photolytic product of DACP and 1-MCP show competitive kinetics with ethylene before the compound is bound. After these "permanent" blocking compounds have bound, competition has not been demonstrated because the receptor has been inactivated for too long. In ripening fruit even large amounts of ethylene (e.g. $1,000 \text{ } \mu\text{l.l}^{-1}$) do not give a response after these compounds have bound. The difference in the two groups seems

Bound to the Receptor?

In tobacco leaves half of the bound ethylene diffuses out in 10 minutes. In tomato leaves, half appears to diffuse out in less than 2 minutes. In some cases, ethylene diffuses from the binding site(s) in two or three phases, depending on the plant material (Sisler, 1991; Sanders et al., 1991). In mung bean sprouts, there is a rapid phase with a half-life of from 8_10 minutes. Another lasts about 1.5_2 h, and another from 10_24 h. Growth experiments (Warner and Leopold, 1971) in pea suggest the short time site is a receptor.

Diffusion of ethylene blocking compounds from mung bean sprouts has been measured in a few cases (Table 2). 2,5-NBD diffuses away with a half-life of 3 h, and *trans*-cyclooctene diffuses away with a half life of 6 h. Both are compounds that require continuous exposure. The time constants of compounds omit which bind for long periods of time have not been measured, because the tissue usually has changed during that time interval. Some tissues require 12 days to become sensitive to ethylene again. When tissues are treated with other compounds they may require less time to become sensitive again; 7 days in the case of 3,3-DMCP and 5 days for others (Sisler et al., unpublished). These widely divergent times to regain activity suggest that the same receptor is becoming active again rather than that new receptors are being produced, although both may be partly responsible. It probably can be assumed that these periods represent, roughly, the time required for half of the receptor to be free.

Why do Olefins Bind to Metals?

Burg and Burg (1967) reported that the concentration needed for the biological activity of olefins in an

to be that the "permanent" blocking agents dissociate too slowly to demonstrate competition. However plant material does become sensitive to ethylene after 12 days at 25°C, and some dissociation is thought to take place. It is possible that new receptors are made, but tissues treated with 3,3-dimethylcyclopropene (3,3-DMCP) become sensitive to ethylene within 7 days after treatment, suggesting that the receptor becomes free. It would thus

ethylene response was of the same order as those compounds that bind to silver (Muhs and Weiss, 1962). Binding to silver is reported to be of the same order as ring strain (Traynham and Olechowski, 1959). All double bonds have inherent

Table 2. Time required for the receptor to become free. Time is for 1/2 of the receptor to become free after being exposed to the compound. In the case of 3,3-DMCP and 1-MCP, time refers to time for bananas become sensitive after a single exposure to ethylene.

Compound	Plant	Time (Minutes)
Ethylene	Mung bean sprout	10
Ethylene	Tobacco leaf	10
Ethylene	Tomato leaf	2
2,5-NBD	Mung bean sprout	180
Trans-cyclooctene	Mung bean sprout	360
3,3-DMCP	Banana	25,200
1-MCP	Banana	43,200

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strain (Wiberg, 1987). Even ethylene may be looked at as having considerable inherent strain, which can be relieved by accepting electrons. Some other olefins have considerably more strain and accept electrons to relieve that strain. The greater the strain, the tighter the binding and presumably the longer the bonds would last, unless some other factor such as steric effects came into play.

What are the Ligands?
Exactly what ligands are is unknown, but in proteins groups such as histidine, methionine, thiol groups, tyrosine groups, and, in a hydrophobic environment such as a membrane, perhaps the double bonds in unsaturated fatty acids could all be ligands. The ligands could be on a single protein or perhaps form a cross link between

How do these Compounds Prevent Ethylene Action?

When ethylene binds to the receptor, it must do something which the blocking compounds do not do, and these compounds must also prevent ethylene from doing it. Molecular size could be involved, but 1-MCP and methylenecyclopropane have the same empirical formula and differ only in the position of the double bond (Figure 4). 1-MCP is an ethylene antagonist while methylenecyclopropane is an agonist. They should be similar in size. Chatt et al. (1955) have proposed that binding of compounds such as ethylene to metals makes the position *trans* to the bound ethylene more susceptible to substitution by other ligands by withdrawing electrons from the metal and giving the metal more affinity for electrons in the opposite position. The process probably has at least two steps: In Step 1, both those compounds which induce a response and those that block the receptor should bind to the supposed metal and withdraw electrons from the metal. This would likely result in a rearrangement or change in ligands on the metal. In Step 2, ethylene likely dissociates from the metal (or is pushed off) in a few minutes while those that block receptors appear to take at least several hours to leave, and some many days (Table 2). The leaving time from the metal seems to be the thing that is different. If high levels of compound are used, binding time is rapid (a few minutes), which rules it out as the controlling factor. It would thus appear that ethylene leaving the complex causes the complex to become active probably by further ligand rearrangement since those that do not leave rapidly do not form an active complex.

two proteins. A small molecule could also supply a ligand. It has been shown (Schaller and Bleecker, 1995) that replacing a cysteine or histidine (Schaller et al., 1997) eliminates ethylene responses in *Arabidopsis* and eliminates ethylene binding in yeast. This suggests that cysteine and histidine are ligands although the function of cysteine could be to put a metal in a low oxidation state, causing it to bind ethylene in the hydrophobic environment. The receptor complex probably needs to be isolated from the plant tissue and the binding site located by radioactive label to determine with certainty what the metal is and to what it is bound. Even this approach may be difficult if the metal in the receptor is labile.

How does the Active Complex Work?

At this time there is no information on how the active complex works except that ethylene responses are manifest. We know that compounds which induce responses also accept electrons, and those that block are those acceptors that are strained. This does not tell us how a ligand substitution process turns on the signal transduction pathway. This problem needs to be addressed in future research.

Where is the Site of Ethylene Action?

Recent molecular biology studies indicate the gene product of ETR1 as the site of ethylene action (Schaller and Bleecker, 1995). When ETR1 is cloned in yeast, it binds ethylene. Ethylene diffuses from this site with a $t_{1/2}$ of 12.5 h. The major binding component in *Arabidopsis* diffuses from the binding site rapidly (Sanders et al., 1991) unless the plants are incubated with ^{14}C -ethylene many hours when another binding site appears from which diffusion is

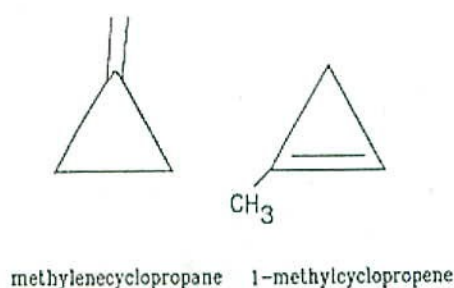


Figure 4. Structure of 1-MCP and methylenecyclopropane. 1-MCP is a very effective antagonist of ethylene action, and methylenecyclopropene is an ethylene agonist.

much slower. Plants have a number of ethylene binding sites. Mung bean seeds bind large amounts of ethylene, far in excess of vegetative tissue, and most of it dissociates over a period of many hours. Inorganic Cu^+ binds ethylene in aqueous suspension, and it has a very definite dissociation pressure. It must be realized that the discrepancy in dissociation times for ethylene in yeast and in *Arabidopsis* is a serious discrepancy. The growth evidence of Warner and Leopold (1971) in pea strongly suggest a short time association-dissociation for ethylene in controlling growth. Plants containing a mutant ETR1 gene bind less ethylene than normal plants, and this is the component that seems to disappear in mutant *Arabidopsis* plants (Bleecker et al., 1988). Histidine and cysteine are essential for ethylene binding in this cloned ETR1 protein, and binding is thought to be Cu^+ . Many

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metals in proteins are bound through histidine, and cysteine in a hydrophobic environment would likely reduce Cu^{++} to Cu^+ . This should bind ethylene. Binding alone by this over expressed protein does not mean that the ethylene binding has anything to do with physiological activity. The discrepancy in dissociating time does not rule out this binding being the site of the physiological ethylene response. The discrepancy may mean the Cu^+ is not properly oriented or some other component is missing, and it could mean the metal is different from copper. It should be mentioned that when mung bean sprouts are blended, the

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short time-binding component disappears and does not appear to bind ethylene *in vitro* (Sisler, 1991). This could be due to the loss of the metal, or possibly the change of valence by the metal when it is exposed to high oxygen levels or exposed to water thus releasing bound ethylene. It will be necessary to show that the ethylene binding in ETR1 has physiological activity before it can be said on that basis that ETR1 is the receptor, although the evidence does favor ETR1 as being at least part of the receptor.

Future Work

Despite the vast amount of work done in the past, some problems remain. Perhaps the biggest are identifying the supposed metal in the receptor, finding to what ligands it is bound and where they are located. Other questions are: Is there more than one type of receptor? How does ethylene turn the ethylene response pathway on, and how is it regulated? Still other challenges include better control of these responses and extending the shelf life of fruits and vegetables using these materials.

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United States Patent [19]

Daly et al.

[11] Patent Number: 6,017,849

[45] Date of Patent: Jan. 25, 2000

[54] SYNTHESIS METHODS, COMPLEXES AND DELIVERY METHODS FOR THE SAFE AND CONVENIENT STORAGE, TRANSPORT AND APPLICATION OF COMPOUNDS FOR INHIBITING THE ETHYLENE RESPONSE IN PLANTS

[75] Inventors: James Daly; Bob Kourelis, both of Chicago, Ill.

[73] Assignee: Biotechnologies for Horticulture, Inc., Burr Ridge, Ill.

[21] Appl. No.: 09/137,056

[22] Filed: Aug. 20, 1998

[51] Int. Cl.⁷ A01N 33/04; A01N 3/02; A01N 25/08; A01N 25/18; A01N 27/00; A01N 29/04

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[58] Field of Search 504/114, 115, 504/320, 326, 353, 356; 585/23, 365, 379, 380

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Article entitled: "STS: Still the Best Weapon in the Ethylene Battle", *Link Magazine*, Aug. 1993, pp. 35-37.

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[57] ABSTRACT

The present invention generally relates to the regulation of plant physiology, in particular to methods for inhibiting the ethylene response in plants or plant products, and has three embodiments. The first embodiment relates to methods of minimizing impurities capable of reversibly binding to plant ethylene receptor sites during the synthesis of cyclopropene and its derivatives such as methylcyclopropene, thereby avoiding the negative effects these impurities have on plants treated with cyclopropene and its derivatives. The second embodiment relates to complexes formed from molecular encapsulation agents such as cyclodextrin, and cyclopropene and its derivatives such as methylcyclopropene, in addition to cyclopentadiene and diazocyclopentadiene and their derivatives, thereby providing a convenient means for storing and transporting these compounds capable of inhibiting the ethylene response in plants, which are reactive gases and highly unstable because of oxidation and other potential reactions. The third embodiment relates to convenient methods of delivering to plants these compounds capable of inhibiting the ethylene response in the plants in order to extend their shelf life.

26 Claims, No Drawings

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SYNTHESIS METHODS, COMPLEXES AND DELIVERY METHODS FOR THE SAFE AND CONVENIENT STORAGE, TRANSPORT AND APPLICATION OF COMPOUNDS FOR INHIBITING THE ETHYLENE RESPONSE IN PLANTS

FIELD OF THE INVENTION

The present invention generally relates to the regulation of plant physiology, in particular to methods for inhibiting the ethylene response in plants or plant products, in order to prolong their shelf life. The invention relates to prolonging the shelf life of cut flowers and ornamentals, potted plants (edible and non-edible), transplants, and plant foods including fruits, vegetables and root crops.

The present invention has three embodiments. The first embodiment relates to methods of minimizing impurities capable of reversibly binding to plant ethylene receptor sites during the synthesis of cyclopropene and its derivatives, in particular methylcyclopropene. Certain impurities produced during the manufacture of cyclopropene and its derivatives, in particular methylcyclopropene, have negative effects on treated plants. Therefore, when plants are treated with cyclopropene and its derivatives, in particular methylcyclopropene, made by using the methods of synthesis of the present invention, the negative effects of these impurities are avoided.

The second embodiment of the present invention relates to complexes formed from molecular encapsulation agents, such as cyclodextrin, and cyclopropene or its derivatives, such as methylcyclopropene, in addition to complexes formed from molecular encapsulation agents and cyclopentadiene or diazocyclopentadiene or their derivatives. These molecular encapsulation agent complexes provide a convenient and safe means for storing and transporting the compounds capable of inhibiting the ethylene response in plants. These molecular encapsulation agent complexes are important because the compounds capable of inhibiting the ethylene response in plants are reactive gases and therefore highly unstable because of oxidation and other potential reactions.

The third embodiment relates to convenient methods of delivering to plants the compounds capable of inhibiting their ethylene responses in order to extend shelf life. These methods involve contacting the molecular encapsulation agent complex with a solvent capable of dissolving the molecular encapsulation agent, thereby liberating the compound capable of inhibiting the ethylene response so it can contact the plant.

BACKGROUND OF THE INVENTION

The present invention generally relates to the regulation of plant growth and to methods of inhibiting ethylene responses in plants by application of cyclopropene, cyclopentadiene, diazocyclopentadiene or their derivatives, in particular methylcyclopropene. The present invention specifically relates to methods of synthesis and molecular encapsulation agent complexes, in addition to storage, transport and application of these gases that inhibit ethylene responses in plants.

Plant growth responses are affected by both internal and external factors. Internal control of plant processes are under the influence of genetic expression of the biological clocks of the plant. These processes influence both the extent and timing of growth processes. Such responses are mediated by signals of various types which are transmitted within and

between cells. Intracellular communication in plants typically occurs via hormones (or chemical messengers) as well as other less understood processes.

Because communications in a plant are typically mediated by plant hormones, both the presence and levels of such hormones are important to specific plant cell reactions. The plant hormone that is most relevant to the present invention is ethylene, which has the capacity to affect many important aspects of plant growth, development and senescence. The most important effects of ethylene include processes normally associated with senescence, particularly fruit ripening, flower fading and leaf abscission.

It is well known that ethylene can cause the premature death of plants including flowers, leaves, fruits and vegetables. It can also promote leaf yellowing and stunted growth as well as premature fruit, flower and leaf drop.

Because of these ethylene-induced problems, very active and intense research presently concerns the investigation of ways to prevent or reduce the deleterious effects of ethylene on plants.

One major type of treatment used to mitigate the effects of ethylene employs ethylene synthesis inhibitors. These ethylene synthesis inhibitors reduce the quantity of ethylene that a plant can produce. Specifically, these ethylene synthesis inhibitors inhibit pyridoxal phosphate-mediated reactions and thereby prevent the transformation of S-adenosylmethionine to 1-amino cyclopropane-1-carboxylic acid, the precursor to ethylene. Staby et al. ("Efficacies of Commercial Anti-ethylene Products for Fresh Cut Flowers", Hort Technology, pp. 199-202, 1993) discuss the limitations of these ethylene synthesis inhibitors. Because ethylene synthesis inhibitors only inhibit a treated plant's production of ethylene, they do not suppress the negative effects of ethylene from environmental sources. These environmental sources of ethylene exist because ethylene is also produced by other crops, truck exhaust, ethylene gas units and other sources, all of which can affect a plant during production, shipment, distribution and end use. Because of this, ethylene synthesis inhibitors are less effective than products that thwart a plant's ethylene responses. For a discussion of the ethylene response in plants, see U.S. Pat. No. 3,879,188.

The other major type of treatment used to mitigate the effects of ethylene employs blocking the receptor site that signals ethylene action. One of the best known compounds for inhibiting the ethylene response in plants, as well as preventing the deleterious effects from environmental sources of ethylene, is silver thiosulfate ("STS"). An example of a commercial STS product is SILFLOR solution, available from Floralife, Inc., Burr Ridge, Ill. STS is very effective in inhibiting the ethylene response in plants and has been used because it moves easily in the plant and is not toxic to plants in its effective concentration range. STS can be used by growers, retailers and wholesalers as a liquid that is absorbed into the stems of the flowers. While STS is highly effective, it has a serious waste disposal problem. It is illegal to dispose of the silver component of STS by conventional means, such as by using a laboratory sink, without first pretreating the STS to remove the silver. It is also illegal to spray STS on potted plants. Consequently because of this disposal problem which is typically ignored by growers, STS is now almost exclusively utilized only by growers. Therefore, there is a great desire among postharvest physiologists to find alternatives to STS. To the knowledge of the present inventors, the only commercially acceptable replacements for STS are cyclopropene, cyclopentadiene, diazocyclopentadiene and their derivatives.

Many compounds such as carbon dioxide which block the action of ethylene diffuse from the ethylene receptor or binding site over a period of a few hours. Sisler & Wood, Plant Growth Reg. 7, 181-191, 1988. While these compounds may be used to inhibit the action of ethylene, their effect is reversible and therefore they must be exposed to the plant in a continuous manner if the ethylene inhibition effect is to last for more than a few hours. Therefore, an effective agent for inhibiting the ethylene response in plants should provide an irreversible blocking of the ethylene binding sites and thereby allow treatments to be of short duration.

An example of an irreversible ethylene inhibiting agent is disclosed in U.S. Pat. No. 5,100,462. However, the diazo-cyclopentadiene described in that patent is unstable and has a strong odor. Sisler et al., Plant Growth Reg. 9, 157-164, 1990, showed in a preliminary study that cyclopentadiene was an effective blocking agent for ethylene binding. However, the cyclopentadiene described in that reference is also unstable and has a strong odor.

U.S. Pat. No. 5,518,988 discloses the use of cyclopropene and its derivatives, including methylcyclopropene, as effective blocking agents for ethylene binding. Although the compounds in this patent do not suffer from the odor problems of diazocyclopentadiene and cyclopentadiene, because they contain a carbene group, they are relatively unstable due to their potential for undergoing oxidation and other reactions. Therefore, a problem of stability of these gases, as well as the explosive hazards these gases present when compressed, exist. To solve these problems, the present inventors have developed a method of incorporating these gaseous compounds, which inhibit the ethylene response in plants, in a molecular encapsulation agent complex in order to stabilize their reactivity and thereby provide a convenient and safe means of storing, transporting and applying or delivering the active compounds to plants. The application or delivery methods of these active compounds can be accomplished by simply adding water to the molecular encapsulation agent complex.

In trying to implement the teaching of U.S. Pat. No. 5,518,988, the problems associated with the stability of the gases and the potential explosive hazard of using compressed gases limit their use and therefore their effectiveness. To solve those problems, the present inventors developed a molecular encapsulation agent complex that stabilizes the reactivity of these gases and thereby provides a convenient and safe means of storing, transporting and applying or delivering these gases to plants.

This approach is an important advance over the art as it allows for the convenient and safe storage, transport and use of gases that are otherwise difficult to store, ship and dispense. The present invention will now allow for the safe, convenient and consistent use of these gases in the field by the grower, in addition to their use in distribution and in the retail marketplace. In fact, a complex of methylcyclopropene and the molecular encapsulating agent cyclodextrin allows for a product having a shelf life of greater than one year.

Another feature of the molecular encapsulation agents of the present invention is that once they trap the gaseous active agent in the complex, the complex (and hence the gaseous active agent) does not exhibit a very high vapor pressure and is therefore protected from oxidation and other chemical degradation reactions. A gaseous active compound such as cyclopropene or derivatives thereof is held in a caged molecule whereby the vapor pressure of the solid is very low due to the weak atomic forces (van de Waals and hydrogen

binding). The binding of these gaseous active compounds with these molecular encapsulation agents holds the active compound until ready for use.

The present invention also prolongs the life of plants by providing an effective and proper dose of the encapsulated active compound capable of inhibiting the ethylene response, which is subsequently desorbed into a gas form for administration to the plant. The invention further embodies the release of the desired active compound from the complex by dissolving the complex in a suitable solvent in order to release the gaseous active compound, thereby serving as an improved gaseous plant treatment.

A major advantage of the present invention is that it provides an effective, user-friendly product for non-technical customers, florists and wholesalers. In addition, the molecular encapsulation agent complex acts as a controlled release agent for treatment with such active gaseous compounds as cyclopropene and methylcyclopropene. As a result, the present invention promotes less human exposure to the target compound than other means of application. Additionally, the user has more control over the application of the gaseous active compound because the active gaseous compound is slowly released from the complex in the presence of a suitable solvent.

Another advantage of the present invention is the amount of selective inclusion of the gaseous active compounds such as cyclopropene and methylcyclopropene into the molecular encapsulation agent. Using the teachings of the present invention, significant quantities of methylcyclopropene and other active compounds can now be encapsulated into a molecular encapsulation agent such as cyclodextrin, far exceeding the normal expected amount usually found with other solids.

A still further advantage of the present invention over the use of compressed concentrated gases is the elimination of the need for gas tanks, regulators, and OSHA compliance for pressurized gas tanks. This results in a substantial cost savings for the manufacturer as well as the customer. In addition, it eliminates the explosive and flammable potential associated with the use of gas tanks holding a highly reactive organic molecule. Moreover, the present invention eliminates the self polymerization and decomposition of gases that occur with compressed gases or liquids containing them.

Another advantage of the present invention over other inert solid carrier systems proposed for use in applying cyclopropene, such as dust, talc, silica and flour, is that it provides a product containing the active gaseous compound with increased stability. For example, the molecular encapsulation agent cyclodextrin protects the active cyclopropene or methylcyclopropene molecule from external conditions, such as ultraviolet degradation, which are problematic in photosensitive compounds such as these.

A still further advantage of the present invention is that this molecular encapsulation agent complex results in more effective use of the active gaseous compound. For example, a reduced quantity of cyclopropene can be utilized to obtain an effective treatment compared with the use of prior proposed cyclopropene solid carriers or compressed gases. This results in less waste and less packaging needed for the commercial product.

In another embodiment, this invention relates to the synthesis of cyclopropene and its derivatives including methylcyclopropene by methods that lower the incidence of impurities, such as hazardous reaction products and by-products, that interfere with the ethylene binding effec-

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tiveness of cyclopropene and its derivatives. These reaction product impurities include compounds that bind tightly but reversibly to the ethylene receptor site and inhibit the irreversible binding of cyclopropene and its derivatives, especially methylcyclopropene. The synthesis of these cyclopropene and derivative compounds is important because if irreversible binding to the receptor site does not take place during plant treatment, the plant will not be protected against the effects of ethylene.

The prior art syntheses of methylcyclopropene has created problems when the methylcyclopropene was used for inhibiting the ethylene response in plants. While it is well documented in U.S. Pat. No. 5,518,988 that methylcyclopropene and other similar compounds are active against ethylene, it has been discovered that not all methods of synthesis are as effective or preferable as the presently claimed synthesis method.

First, it is necessary to avoid producing during synthesis products (or impurities) that reversibly bind to the same ethylene receptor site as the intended active compound. Because these impurities do not irreversibly bind in a manner consistent with the inactivation of the receptor site without phytotoxicity, the effectiveness of using such a reaction product mixture without further processing is reduced. The specific impurities that must be avoided in the synthesis in order to obtain optimal performance of the reaction mixture include methylenecyclopropane, methylcyclopropanes and butanes.

The present inventors have discovered that of all the Lewis bases used for the production of methylcyclopropene, sodium amide and lithium diisopropylamide are most preferred. Synthesis using various metal hydrides and hydroxides were found to produce high levels of other reaction products that lowered the performance of the methylcyclopropene for plant uses. For example, using butynes, 3-hydroxy-2-methylpropenes and other similar starting materials generally yields an impure reaction product that is not appropriate for use in the treatment of plants.

Additional features and advantages of the present invention are described in, and will be apparent from, the detailed description and examples provided.

SUMMARY OF THE INVENTION

In a method of minimizing impurities embodiment, the present invention relates to a method of minimizing impurities capable of reversibly binding to plant ethylene receptor sites comprising the steps of reacting, in an inert environment, a metal amide salt and a halogenated carbene, optionally in the presence of a non-reactive solvent, to form a compound having the following structure



wherein n is a number from 1 to 4 and R is selected from the group consisting of hydrogen, saturated or unsaturated C1 to C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino and carboxy. This method of minimizing impurities embodiment is generically referred to as the cyclopropene method of minimizing impurities. The preferred metal amide salts for use in this method of minimizing impurities embodiment are sodium amide, lithium amide, potassium amide, lithium diisopropylamide and sodium diisopropylamide. The preferred halogenated carbenes for use in this method of minimizing impurities embodiment are 3-chloro-3-methyl-

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2-methylpropene, 3-bromo-3-methyl-2-methylpropene, 3-chloro-2-methylpropene and 3-bromo-2-methylpropene.

In a more specific method of minimizing impurities embodiment, the present invention relates to a method of minimizing impurities capable of reversibly binding to plant ethylene receptor sites comprising the steps of reacting, in an inert environment, a metal amide salt and a halogenated methyl propene, optionally in the presence of a non-reactive solvent, to form methylcyclopropene. This more specific method of minimizing impurities embodiment is referred to as the methylcyclopropene method of minimizing impurities. The preferred metal amide salts for use in this more specific method of minimizing impurities embodiment are sodium amide, lithium amide, potassium amide, lithium diisopropylamide and sodium diisopropylamide. The preferred halogenated methyl propenes for use in this more specific method of minimizing impurities embodiment are 3-chloro-2-methylpropene and 3-bromo-2-methylpropene.

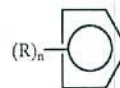
In one of the molecular encapsulation agent complex embodiments, which is generically referred to as the cyclopropene molecular encapsulation agent complex, the complex is formed from a molecular encapsulation agent and a compound having the following structure



wherein n is a number from 1 to 4 and R is selected from the group consisting of hydrogen, saturated or unsaturated C1 to C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino and carboxy. The preferred molecular encapsulation agents for use in this cyclopropene molecular encapsulation agent complex embodiment include a cyclodextrin, a crown ether, a polyoxyalkylene, a phosphorine, a polysiloxane, a phosphazene and a zeolite. Cyclodextrin and in particular alpha-cyclodextrin are particularly preferred. The preferred compounds capable of inhibiting the ethylene response in plants for use in this cyclopropene molecular encapsulation agent complex embodiment are cyclopropene and dimethylcyclopropene.

In a more specific molecular encapsulation agent complex embodiment, which is referred to as the methylcyclopropene molecular encapsulation agent complex, the complex is formed from a molecular encapsulation agent and methylcyclopropene. The preferred molecular encapsulation agents for use in this methylcyclopropene molecular encapsulation agent complex embodiment include a cyclodextrin, a crown ether, a polyoxyalkylene, a phosphorine, a polysiloxane, a phosphazene and a zeolite. Cyclodextrin and in particular alpha-cyclodextrin are particularly preferred.

In another molecular encapsulation agent complex embodiment, which is generically referred to as the cyclopentadiene molecular encapsulation agent complex, the complex is formed from a molecular encapsulation agent and a compound having the following structure

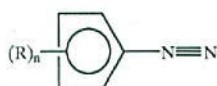


wherein n is a number from 1 to 4 and R is selected from the group consisting of hydrogen, saturated or unsaturated C1 to C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino and carboxy. The preferred molecular encapsulation agents for use in this cyclopentadiene molecular encapsulation agent

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complex embodiment include a cyclodextrin, a crown ether, a polyoxyalkylene, a prophorine, a polysiloxane, a phop-hazene and a zeolite. Cyclodextrin and in particular alpha-cyclodextrin are particularly preferred.

In still another molecular encapsulation agent complex embodiment, which is generically referred to as the diazo-cyclopentadiene molecular encapsulation agent complex, the complex is formed from a molecular encapsulation agent and a compound having the following structure



wherein n is a number from 1 to 4 and R is selected from the group consisting of hydrogen, saturated or unsaturated C1 to C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino and carboxy. The preferred molecular encapsulation agents for use in this diazocyclopentadiene molecular encapsulation agent complex embodiment include a cyclodextrin, a crown ether, a polyoxyalkylene, a prophorine, a polysiloxane, a phop-hazene and a zeolite. Cyclodextrin and in particular alpha-cyclodextrin are particularly preferred.

In one of the method of delivery of a compound to a plant to inhibit an ethylene response in the plant embodiments, which is generically referred to as the cyclopropene method of delivery, the method comprises the step of contacting a complex formed from a molecular encapsulation agent and a compound having the following structure



wherein n is a number from 1 to 4 and R is selected from the group consisting of hydrogen, saturated or unsaturated C1 to C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino and carboxy, with a solvent capable of dissolving the molecular encapsulation agent, and thereby liberating the compound from the molecular encapsulation agent so that it can contact the plant. The preferred molecular encapsulation agents for use in this cyclopropene method of delivery embodiment include a cyclodextrin, a crown ether, a polyoxyalkylene, a prophorine, a polysiloxane, a phop-hazene and a zeolite. Cyclodextrin and in particular alpha-cyclodextrin are particularly preferred. The preferred compounds capable of inhibiting the ethylene response in plants for use in this cyclopropene method of delivery embodiment are cyclopropene and dimethylcyclopropene. The preferred solvent for use in this cyclopropene method of delivery embodiment is water, and the water may additionally comprise an acidic or alkaline agent. A more specific feature of this cyclopropene method of delivery embodiment comprises bubbling a gas through the solvent while it is in contact with the complex. In addition, another specific feature of this cyclopropene method of delivery embodiment comprises applying heat to the solvent either before it contacts the complex or during that contact.

In a more specific method of delivery embodiment, which is specifically referred to as the methylcyclopropene method of delivery, the method comprises the step of contacting a complex formed between a molecular (encapsulation agent and methylcyclopropene with a solvent capable of dissolving the molecular encapsulation agent, and thereby liberating the methylcyclopropene from the molecular encapsulation agent so that it can contact the plant. The preferred

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molecular encapsulation agents for use in this methylcyclopropene method of delivery embodiment include a cyclodextrin, a crown ether, a polyoxyalkylene, a prophorine, a polysiloxane, a phop-hazene and a zeolite. Cyclodextrin and in particular alpha-cyclodextrin are particularly preferred. The preferred solvent for use in this methylcyclopropene method of delivery embodiment is water, and the water may additionally comprise an acidic or alkaline agent. For example, a buffering solution that can be used to facilitate the release of the methylcyclopropene gas contains 0.75% potassium hydroxide and 0.75% sodium hydroxide after the proper amount of water is added. A more specific feature of this methylcyclopropene method of delivery embodiment comprises bubbling a gas through the solvent while it is in contact with the complex. In addition, another specific feature of this methylcyclopropene method of delivery embodiment comprises applying heat to the solvent either before it contacts the complex or during that contact.

In another method of delivery embodiment, which is generically referred to as the cyclopentadiene method of delivery, the method comprises the step of contacting a complex formed from a molecular encapsulation agent and a compound having the following structure



wherein n is a number from 1 to 4 and R is selected from the group consisting of hydrogen, saturated or unsaturated C1 to C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino and carboxy, with a solvent capable of dissolving the molecular encapsulation agent, and thereby liberating the compound from the molecular encapsulation agent so that it can contact the plant. The preferred molecular encapsulation agents for use in this cyclopentadiene method of delivery embodiment include a cyclodextrin, a crown ether, a polyoxyalkylene, a prophorine, a polysiloxane, a phop-hazene and a zeolite. Cyclodextrin and in particular alpha-cyclodextrin are particularly preferred. The preferred solvent for use in this cyclopentadiene method of delivery embodiment is water, and the water may additionally comprise an acidic or alkaline agent. A more specific feature of this cyclopentadiene method of delivery embodiment comprises bubbling a gas through the solvent while it is in contact with the complex. In addition, another specific feature of this cyclopentadiene method of delivery embodiment comprises applying heat to the solvent either before it contacts the complex or during that contact.

In still another method of delivery embodiment, which is generically referred to as the diazocyclopentadiene method of delivery, the method comprises the step of contacting a complex formed from a molecular encapsulation agent and a compound having the following structure



wherein n is a number from 1 to 4 and R is selected from the group consisting of hydrogen, saturated or unsaturated C1 to C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino and carboxy, with a solvent capable of dissolving the molecular encapsulation agent, and thereby liberating the compound

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from the molecular encapsulation agent so that it can contact the plant. The preferred molecular encapsulation agents for use in this diazocyclopentadiene method of delivery embodiment include a cyclodextrin, a crown ether, a polyoxyalkylene, a prophorine, a polysiloxane, a phop-
hazene and a zeolite. Cyclodextrin and in particular alpha-cyclodextrin are particularly preferred. The preferred solvent for use in this diazocyclopentadiene method of delivery embodiment is water, and the water may additionally comprise an acidic or alkaline agent. A more specific feature of this diazocyclopentadiene method of delivery embodiment comprises bubbling a gas through the solvent while it is in contact with the complex. In addition, another specific feature of this diazocyclopentadiene method of delivery embodiment comprises applying heat to the solvent either before it contacts the complex or during that contact.

DETAILED DESCRIPTION OF THE INVENTION

The Compounds that Inhibit Plant Ethylene Responses

The compounds that inhibit ethylene responses in plants are disclosed in the following references, all of which are incorporated by reference. U.S. Pat. No. 5,100,462 discloses that diazocyclopentadiene and its derivatives are effective blocking agents that inhibit the ethylene response in plants. Sisler et al., Plant Growth Reg. 9, 157-164, 1990, discloses that cyclopentadiene was an effective blocking agent for inhibiting the ethylene response in plants. U.S. Pat. No. 5,518,988 discloses that cyclopropene and its derivatives, including methylcyclopropene, are effective blocking agents for inhibiting the ethylene response in plants. Rather than repeat the disclosure of those references in this specification, they are incorporated by reference in their entireties.

The derivatives of cyclopropene, cyclopentadiene and diazocyclopentadiene may contain from 1 to 4 R groups. The number of such R groups is more preferably 2 and most preferably 1. As previously mentioned, suitable R groups include hydrogen, saturated or unsaturated C1 to C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino and carboxy. The term "alkyl" is defined herein to refer to linear or branched, saturated or unsaturated alkyl groups. Examples include but are not limited to methyl, ethyl, propyl, isopropyl and butyl. Alkyl groups of the present invention are most preferably single carbon or linear.

The Synthesis of the Cyclopropene and Methylcyclopropene Embodiments

Pursuant to the present invention, cyclopropene and its derivatives are made by reacting, in an inert environment, a metal amide salt, such as lithium amide salt, sodium amide salt, potassium amide salt, lithium diisopropylamide salt, sodium diisopropylamide salt or other metal amide salts, and a halogenated carbene, such as 3-chloro-3-methyl-2-methylpropene, 3-bromo-3-methyl-2-methylpropene, 3-chloro-2-methylpropene, 3-bromo-2-methylpropene or some other halogenated carbene. The specific compounds named above are preferred. Methylcyclopropene is made under the same conditions with the same metal amide salts discussed above by reacting them with a halogenated methylpropene. The preferred halogenated methyl propenes are 3-chloro-2-methylpropene and 3-bromo-2-methylpropene. These halogenated methyl propenes lead to a high purity product for the intended use and are readily available.

Suitable methods for making cyclopropene and its derivatives, including methylcyclopropene, are covered in the examples below. While a variety of different volatile and non-volatile non-reactive solvents can be utilized, preferred suitable solvents include glycerine, mineral oil, polyethylene glycol, diglyme and tetraglyme. The use of a non-

reactive solvent is optional. The inert environment can be created by any known method including purging the reaction vessel with nitrogen or any other inert gas.

The concentration ratio of the metal amide salt to this halogenated carbene or halogenated methyl propene is a molar ratio of about 1:1 to about 4:1. The reaction temperature can range from about 20° to about 60° C. and the reaction pressure can range from about 1 to about 100 psi.

The resulting exothermic solution from this reaction is allowed to react until no further heat is given off. After the reaction is complete, a polar solvent is added to the reaction solution. While a variety of polar solvents can be used, suitable examples of such polar solvents include water, acetone and alcohol. After the polar solvent has been added, the head space of the reaction solution is displaced, cooled and placed into a second vessel containing a molecular encapsulation agent, such as cyclodextrin, and buffered water to form the desired molecular encapsulation agent complex.

When the gas is released into the original vessel using sodium amide, a non-polar solvent is used to release the gas when a lithium salt is employed as the metal amide salt.

Although it is not necessary to achieve the objectives of this invention, fractional distillation can be used on the final product.

In one preferred embodiment, the headspace of the reaction solution is cooled through a condenser and cold trap. The water used with the molecular encapsulation agent is buffered to approximately a pH of 4 to 6, and the reaction product and molecular encapsulation agent is stirred for 1 to 24 hours at temperatures ranging from room temperature to 40° C. After the complex is formed, the excess water is filtered off and the resulting slurry dried to form a powder. The examples below describe a method of preparing a molecular encapsulation agent from methylcyclopropene and alpha-cyclodextrin.

The Molecular Encapsulation Agent Complex

As previously explained, forming a complex from the molecular encapsulation agent and the gaseous compound capable of inhibiting the ethylene response in plants is important for two reasons. First, strained carbenes such as methylcyclopropene are quite unstable to reaction with oxygen, self polymerization and reaction with other organic compounds. The complexes of the present invention overcome those instability problems. Second, it is preferable to use a product that has a long shelf life, is simple to handle and comparatively non-reactive. The complexes of the present invention meet those objectives as well.

Methylcyclopropene is reactive and explosive at concentrations over one percent. Additionally, it is difficult to handle as a gas, requires compression into metal containers or the use of a non-oxygen permeable container. Since for most applications, less than 1 ppm (part per million) and preferably less than 1 ppb (parts per billion) of methylcyclopropene in the atmosphere are required, the amount of methylcyclopropene required to treat a normal room is about one gram or less. The recommended dosage is around 500-700 ppb for 4-6 hours at room temperature for a few crops.

A molecular encapsulation agent is a compound that has a lock and key structure similar to an enzyme whereby a substrate selectively fits into the encapsulation site.

The most preferred molecular encapsulation agent found to date is alpha-cyclodextrin. Other molecular encapsulation agents, such as crown ethers, polyoxyalkylenes, prophorines, polysiloxanes, phop hazenes and zeolites, were also found to work. Most of these molecular encapsulation agents can be obtained from the Aldrich Chemical Company.

Methylcyclopropene can be complexed with cyclodextrin in water. For example, when the water is removed after methylcyclopropene is bubbled through an aqueous solution of alpha-cyclodextrin, it was discovered that the methylcyclopropene was firmly locked into the cyclodextrin cage structure. In addition, the cyclodextrin cake after drying can be milled into a powder and blended to a uniform concentration. It has been surprisingly discovered that this particular complex (methylcyclopropene and alpha-cyclodextrin) was stable for over one year as judged by accelerated shelf life studies.

Moreover, a powdered complex can be easily measured and packaged into appropriately-sized doses for treatment of plants.

The method of delivery of the present invention provide a user-friendly application. It also promotes a lower initial dose of active compound and a decrease in the need for repeated applications as compared with previously proposed solid carrier systems.

A variety of molecular encapsulation agents may be utilized in the present invention provided they have the correct cage structure to form a molecular trap for the compound capable of inhibiting the ethylene response in plants. Thus, as one skilled in the art would recognize, the use of other molecular encapsulation agents falls within the spirit and scope of the present invention.

Cyclodextrins, also known as "Schardinger Dextrins", are cyclic oligosaccharides composed of glucose units bonded together by alpha 1,4 bonds. The six-membered ring structure is named alpha-cyclodextrin, the seven membered ring is beta-cyclodextrin and the eight membered ring is gamma-cyclodextrin. Generally, compounds that are encapsulated fit inside of the oligosaccharide ring.

As is well known, cyclodextrins are produced from starch of any selected plant variety such as corn, potato, waxy maize and the like. The starch may be modified or unmodified starch derived from cereal or tuber origin and the amylose or amylopectin fractions thereof. The selected starch in aqueous slurry at selected concentration up to about 35% by weight solids is usually liquefied as by gelatinization or treatment with liquefying enzyme such as bacterial alpha-amylase enzymes and then subjected to treatment with a cyclodextrin glucosyl transferase enzyme to form the cyclodextrin.

The amount of the individual alpha, beta and gamma cyclodextrins produced by treating the starch with the glucosyl transferase enzyme will vary depending on the selected starch, selected glucosyl transferase enzyme and processing conditions. The parameters to select for glucosyl transferase enzyme conversion for the desired result in the amount of each individual cyclodextrin to be produced is conventional and well described in the literature. Separation and purification of the cyclodextrin thus obtained is also conventional and well known to those of skill in the art.

In one embodiment, the cyclodextrin utilized in the complex of the present invention is alpha-cyclodextrin. However, as one skilled in the art will appreciate, any cyclodextrin or mixture of cyclodextrins, cyclodextrin polymers as well as modified cyclodextrins can also be utilized pursuant to the present invention. Cyclodextrins are available from American Maize Products Company, Hammond, Ind., as well as other vendors.

In order to form a molecular encapsulation agent complex, the active compound and the molecular encapsulation agent molecules are mixed together in a solution for a period of time sufficient to form the complex. The complex is then removed from the solution and dried. The dried complex is then ready for use.

As noted previously, the resulting complex of the present invention provides a number of advantages to manufacturers as well as ultimate consumers. Due to the ability of the cyclodextrin to entrap a large amount of cyclopropene, the present invention should lower the initial dosage of cyclopropene needed for treatment as compared with previously proposed solid carriers. Likewise, it should decrease the need for repeated treatments of cyclopropene compared with previously proposed solid carriers. The potential of these advantages is demonstrated in the examples below which show the unexpected ability of the complex of the present invention to entrap large quantities of cyclopropene.

A still further advantage of the present invention is the increased stability of the resulting methylcyclopropene/alpha-cyclodextrin complex as compared to compressed gas. Based on heat stability testing, it was determined that when concentrated methylcyclopropene gas was exposed to heat of about 50° C., a 75% to 100% reduction in concentration was observed. When left at room temperature, the concentrated gas lost 30% to 42% of its concentration. On the other hand, when the methylcyclopropene/alpha-cyclodextrin complex of the present invention was exposed to 50° C., only a 38% reduction in the concentration of methylcyclopropene was observed. When left at room temperature, there was no reduction in the concentration of methylcyclopropene from the methylcyclopropene/alpha-cyclodextrin complex.

The present invention also provides a convenient product for commercial use. For example, select quantities of the complex of the present invention can be sealed into a package for retail and wholesale use. In one embodiment, the preferable package is made of polyvinyl alcohol. The inventors have discovered that polyvinyl alcohol increases the efficiency of release, reduces any exposure, and insures proper dosage. When the consumer is ready to use the complex, the consumer may either dissolve the powder in an aqueous solution (e.g., water) and expose the resulting solution to the plant.

Understandably, various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its intended advantages. Therefore, the claims are intended to cover such changes and modifications.

The Controlled Release of Compounds Capable of Inhibiting the Ethylene Response in Plants

Controlled release of methylcyclopropene as well as other compounds capable of inhibiting the ethylene response in plants from a molecular encapsulation agent complex such as cyclodextrin is facilitated by the addition of an excess of water. Addition of an acid or alkaline substance to the water also facilitates a faster release of the active compound. Heating the water also facilitates a faster release of the active compound. Because methylcyclopropene has a high vapor pressure at normal working temperatures from 4 to 25° C., it quickly escapes to the atmosphere. By releasing methylcyclopropene from a complex in water in a closed container or room, the methylcyclopropene diffuses onto the ethylene receptor sites of all the plants within the room. Use of fans or other means to move the air for more suitable equilibration in the chamber is also often useful. Depending on the plant, generally a dose of less than 1 ppm (part per million) or preferably less than 500 ppb (parts per billion) of methylcyclopropene or some other active compound in the atmosphere of the sealed container or room for about 2-6 hours is sufficient to protect the plant or plant product from further ethylene damage.

The Plants Applicable to the Present Invention

The term "plant" is used generically in the present invention to also include woody-stemmed plants in addition to field crops, potted plants, cut flowers, harvested fruits and vegetables and ornamentals. Some of the plants that can be treated by the methods of the present invention are listed below.

Plants treated by the compounds of the present invention that inhibit the ethylene response need to be treated at levels that are below phytotoxic levels. This phytotoxic level varies not only by plant but also by cultivar.

When correctly used, the compounds of the present invention prevent numerous ethylene effects, many of which have been disclosed in U.S. Pat. Nos. 5,518,988 and 3,879,188, both of which are incorporated herein by reference in their entirety. The present invention can be employed to combat numerous plant ethylene responses. Ethylene responses may be initiated by either exogenous or endogenous sources of ethylene. Ethylene responses include, for example, (i) the ripening and/or senescence of flowers, fruits and vegetables, (ii) the abscission of foliage, flowers and fruit, (iii) the prolongation of the life of ornamentals, such as potted plants, cut flowers, shrubbery and dormant seedlings, (iv) the inhibition of growth in some plants such as the pea plant, and (v) the stimulation of plant growth in some plants such as the rice plant.

Vegetables which may be treated by the methods of the present invention to inhibit senescence include leafy green vegetables such as lettuce (e.g., *Lactuca sativa*), spinach (*Spinacia oleracea*) and cabbage (*Brassica oleracea*); various roots such as potatoes (*Solanum tuberosum*), carrots (*Daucus*); bulbs such as onions (*Allium* sp.); herbs such as basil (*Ocimum basilicum*), oregano (*Origanum vulgare*) and dill (*Anethum graveolens*); as well as soybean (*Glycine max*), lima beans (*Phaseolus limensis*), peas (*Lathyrus* sp.), corn (*Zea mays*), broccoli (*Brassica oleracea italica*), cauliflower (*Brassica oleracea botrytis*) and asparagus (*Asparagus officinalis*).

Fruits which may be treated by the methods of the present invention to inhibit ripening include tomatoes (*Lycopersicon esculentum*), apples (*Malus domes tica*), bananas (*Musa sapientum*), pears (*Pyrus communis*), papaya (*Carica papaya*), mangoes (*Mangifera indica*), peaches (*Prunus persica*), apricots (*Prunus armeniaca*), nectarines (*Prunus persica nectarina*), oranges (*Citrus* sp.), lemons (*Citrus limonia*), limes (*Citrus aurantifolia*), grapefruit (*Citrus paradisi*), tangerines (*Citrus nobilis deliciosa*), kiwi (*Actinidia chinensis*), melons such as cantaloupes (*C. cantalupensis*) and musk melons (*C. melo*), pineapples (*Aranea comosus*), persimmon (*Diospyros* sp.) and raspberries (e.g., *Fragaria* or *Rubus ursinus*), blueberries (*Vaccinium* sp.), green beans (*Phaseolus vulgaris*), members of the genus *Cucumis* such as cucumber (*C. sativus*) and avocados (*Persea americana*).

Ornamental plants which may be treated by the methods of the present invention to inhibit senescence and/or to prolong flower life and appearance (such as the delay of wilting), include potted ornamentals and cut flowers. Potted ornamentals and cut flowers which may be treated with the methods of the present invention include azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasansensis*), snapdragons (*Antirrhinum* sp.), poinsettia (*Euphorbia pulcherima*), cactus (e.g., *Cactaceae schlumbergera truncata*), begonias (*Begonia* sp.), roses (*Rosa* sp.), tulips (*Tulipa* sp.), daffodils (*Narcissus* sp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), lily (e.g., *Lilium* sp.), gladiolus (*Gladiolus*

sp.), *Alstroemeria* (*Alstroemeria brasiliensis*), anemone (e.g., *Anemone bland*), columbine (*Aquilegia* sp.), aralia (e.g., *Aralia chinesis*), aster (e.g., *Aster carolinianus*), bougainvillea (*Bougainvillea* sp.), camellia (*Camellia* sp.), bellflower (*Campanula* sp.), cockscomb (*Celosia* sp.), falsecypress (*Chamaecyparis* sp.), chrysanthemum (*Chrysanthemum* sp.), clematis (*Clematis* sp.), cyclamen (*Cyclamen* sp.), freesia (e.g., *Freesia refracta*), and orchids of the family *Orchidaceae*.

Plants which may be treated by the methods of the present invention to inhibit abscission of foliage, flowers and fruit include cotton (*Gossypium* spp.), apples, pears, cherries (*Prunus avium*), pecans (*Carva illinoensis*), grapes (*Vitis vinifera*), olives (e.g., *Olea europaea*), coffee (*Coffea arabica*), snapbeans (*Phaseolus vulgaris*), and weeping fig (*Ficus benjamina*), as well as dormant seedlings such as various fruit trees including apple, ornamental plants, shrubbery, and tree seedlings.

In addition, shrubbery which may be treated according to the present invention to inhibit abscission of foliage include privet (*Ligustrum* sp.), photinea (*Photina* sp.), holly (*Ilex* sp.), ferns of the family *Polypodiaceae*, schefflera (*Schefflera* sp.), aglaonema (*Aglaonema* sp.), cotoneaster (*Cotoneaster* sp.), barberry (*Berberis* sp.), waxmyrtle (*Myrica* sp.), abelia (*Abelia* sp.), acacia (*Acacia* sp.), and bromeliads of the family *Bromeliaceae*.

EXAMPLES

While many of the examples described below are related to the synthesis molecular encapsulation agent complexing and delivery or application of methylcyclopropene to plants, the same synthesis methods have also been found effective for cyclopropene and other cyclopropene derivatives and the same molecular encapsulation agent complexing and delivery or application methods have also been found effective for cyclopropene, cyclopentadiene, diazocyclopentadiene and their derivatives. Methylcyclopropene was used in the examples because it is one of the most active derivatives of cyclopropene that binds to the ethylene receptor site of plants.

Example 1

Synthesis of Methylcyclopropene

At room temperature, nitrogen gas (99.95% pure) is pumped into a nitrogen vessel (35½"x28"x32") containing either sodium amide powder (90%-NaNH₂) or lithium diisopropylamide powder (97%-[$(CH_3)_2CH$]₂NLi). A separate powder addition vessel is also purged with the same nitrogen gas. Purging with nitrogen is necessary because of the reactivity of the above-mentioned Lewis bases with air, and to eliminate any contamination before conducting the synthesis reaction. In the powder addition vessel containing the inert atmosphere, the sodium amide (or an equivalent molar concentration of lithium diisopropylamide) is added in an amount ranging from 365-1100 grams, with the larger amount being preferred. To weigh the proper amount of the Lewis base, all weighing is performed in a nitrogen box with nitrogen purging to eliminate oxygen and the threat of spontaneous ignition of the base. Special care is important when working with such bases for proper safety.

Once the Lewis base in powder form is completely added, the openings in the powder addition vessel that were used for purging are sealed off to exclude air. The powder addition vessel is attached to the main system. The reaction vessel, which already has been purged with nitrogen and has been partially evacuated, is opened to the powder addition vessel to allow the powder to fall into the reaction vessel with the

aid of nitrogen flow. Nitrogen enters the powder addition vessel during transfer of the Lewis base.

After the powder is transferred into the reaction vessel, the ball valve is closed. After the powder is added, a light mineral oil (dried with molecular sieves) or another equivalent solvent is added by opening the connecting ball valve and allowing it to pour into the reaction vessel with the aid of nitrogen flow. The amount of oil added during the reaction can vary from 1-47 liters, with the higher amount 47 liters being preferred. The reaction vessel is then purged and closed. The reaction vessel temperature is adjusted to a temperature anywhere from 0° C. to 75° C., and preferably about 20° C. to start the reaction. The temperature can be raised or lowered by heating or chilling the jacket using a circulating pump. Should the holding capacity of the vessel be exceeded, the procedure is repeated.

During the addition of ingredients, the contents of the reaction vessel are stirred with a propeller mixer, but splashing of the contents should be avoided. After mixing for 1-60 minutes, and preferably for about 20 minutes, 3-chloro-2-methylpropene is added to the reaction vessel in an amount ranging from 0.15-1.0 liters. During the addition of the 3-chloro-2-methylpropene, there is continuous purging with nitrogen gas. The liquid reactant 3-chloro-2-methylpropene is added slowly over a period of 20 minutes. During this addition, the temperature of the reaction vessel is monitored and kept at less than 40° C. Once the 3-chloro-2-methylpropene is completely added, the vessel should be agitated for an additional 1-30 minutes, and preferably for 15 minutes, using the propeller mixer discussed above. A reaction vessel pressure of about two atmospheres is used in this example.

After all the 3-chloro-2-methylpropene has been reacted, the desired end-product, methylcyclopropene, exists as a sodium salt. To react the remainder of the Lewis base and facilitate liberation of the methylcyclopropene product, the nitrogen purge is stopped and water is added ranging from 0.00-1.47 liters by adding the water under positive pressure over a period of 1 hour. Once all the water has been added, a ball valve connecting the vessel with the condenser is opened. Any pressure is then released by bubbling the gaseous methylcyclopropene product through a mixture of cyclodextrin dissolved in water (as explained later in this example).

Once the reactive ingredients have been mixed, the headspace gas in the reaction vessel is transferred to a 5 gallon mixing vessel, already lined with a bag filter (5-25 micron mesh plastic) and containing 0.9-2.8 kg of alpha-cyclodextrin, 0.575 liters of a buffer solution. The alpha-cyclodextrin is weighed out on an electronic scale and transferred to the mixing vessel by pouring it through the opening of the mixing vessel. The buffer solution is prepared by combining a 0.2 M sodium acetate solution with a 0.2 M acetic acid solution which gives a pH in the range of 3 to 5. The headspace gas in the reaction vessel is transferred by pulling a vacuum on the mixing vessel to 15 psi, closing the condenser/reaction vessel ball valve and opening the ball valve linking the condenser (15 coils, 3/8") to the mixing vessel, allowing the gas in the condenser, which has been chilled at a temperature of 0-10° C. by a chilling circulating pump, to pass through to the mixing vessel. The reason for chilling the gas in the condenser is to significantly reduce any 3-chloro-2-methylpropene from entering the mixing vessel. The lower boiling point of methylcyclopropene (which is approximately 12° C.) compared to the higher boiling point of the 3-chloro-2-methylpropene (which is 70° C.) prevents the later from entering the mixing vessel. The

condenser is also positioned in such a way that the 3-chloro-2-methylpropene will return to the reaction flask.

Once the gas passes from the condenser, the condenser/mixing vessel ball valve is closed, and the condenser/reaction vessel ball valve is opened allowing the headspace gas from the reaction vessel to flow into the condenser. The condenser/reaction vessel ball valve is then closed, the condenser/mixing vessel ball valve is reopened, and the gas flows to the mixing vessel. Once the initial head space is transferred over to the mixing vessel, a vacuum will begin to be created in the reaction vessel which can be detected by reading the mounted pressure gauge. When this occurs, the reaction vessel is filled with nitrogen gas (99.95% pure) by closing any connections to the rest of the system, and allowing the nitrogen gas to enter through the nitrogen inlet valve when a slight vacuum occurs. Once the reaction vessel has been filled with nitrogen gas, which will be identifiable by reading the mounted pressure gauge, the head space gas from the reaction vessel is once again transferred to the mixing vessel. The process is repeated until the mixing vessel is filled with gas as indicated by the pressure gauge. A minimum concentration of 80,000 ppm of methylcyclopropene is preferred in the mixing vessel at this step. This concentration can be calculated the same way as previously mentioned. After the mixing vessel is filled, all the connections are closed, and the vessel is removed from the system and placed on a shaker, which is allowed to shake so that the mixture is completely agitated for 1-5 hours at less than 70° C. The methylcyclopropene is trapped in the alpha-cyclodextrin during this unit operation. After the contents are agitated, the mixing vessel is allowed to equilibrate for 0-72 hours, and preferably for at least 24 hours at a temperature of 0-30° C. (preferably about 4° C.). Next, the contents in the mixing vessel, if containing the buffer solution, are filtered out by vacuum filtration, by connecting a vacuum pump at the bottom outlet of the mixing vessel, which will remove the buffer solution from the mixture while the powder remains in the confines of the filtering bag.

Once all the buffer solution has been removed, the wet powder containing the entrapped methylcyclopropene is transferred onto a plastic tray and allowed to air dry for 24-48 hr. Once it has been dried, the filtered material is ground in a powder grinder, creating a fine powder (approximately 100 mm mesh). If the material in the mixing vessel did not contain the buffer solution, no filtering or grinding is needed. After the powder is ground, it is placed in a powder mill and allowed to mix for 5-10° C. minutes at approximately 100 rpm. Once the powder is mixed, it is analyzed and mixed with dextrose or dextrin to the desired concentration of methylcyclopropene entrapment. If the amount of entrapped methylcyclopropene is lower than the desired concentration, it is bulked and milled with other samples. In both cases, after the newly formed powders are mixed, they are analyzed again to insure that they meet specifications. Per every reaction vessel made, 2-7 mixing vessels can be filled, depending on the amount of methylcyclopropene remaining in the reaction vessel after the head space has been transferred. However, depending on the amount of methylcyclopropene gas remaining in the reaction vessel, a waiting period of 0-3 hours may be necessary for the reaction vessel to produce more methylcyclopropene gas. Once the mixing vessels are filled, and there is not enough methylcyclopropene gas to fill more vessels, the reaction vessel is removed from the system, but kept inside a hood.

Cleaning: Water is slowly added to the reaction vessel to begin the cleaning process. Water is added slowly due to its

reactivity with excess sodium amide. When the sodium amide is mixed with water, ammonia and sodium salts are formed. Once the reaction vessel has been washed completely, it is allowed to air dry completely before it is reused. The three addition vessels are cleaned once a week with water. They are thoroughly rinsed with water until no reactants are found. All the piping/tubing and condenser are also cleaned thoroughly once a week with water. The mixing vessels and inner filter linings are thoroughly washed with water after every use. All waste water is disposed of according to governmental regulations. Cleanliness, in addition to purging of the vessels with nitrogen gas and the cooling of gas in the condenser are safety steps that also prevent any contamination of the methylcyclopropene.

Example 2

Manufacture of Methylcyclopropene Using 3-bromo-2-methylpropene and Lithium Diisopropylamide

Under a nitrogen atmosphere, approximately 0.1 to 0.5 moles of lithium diisopropylamide are placed into a two liter container. 100 ml of a non-volatile organic solvent, such as dried mineral oil, is then added to the container. Approximately 0.1 to 0.5 moles of 3-bromo-2-methyl propene is then added to the container. A 1:1 molar ratio of the lithium amide and the halogenated methyl propene is utilized. The exothermic solution is then allowed to react until no heat was given off. Then, approximately 0.1 to 0.5 moles of a polar solvent, such as water, is added to the container.

The head space of the reaction is displaced with a syringe or by sweeping with nitrogen through a condenser and cold trap, connected to a vacuum system into a flask containing approximately 50 to 200 grams of alpha-cyclodextrin and 50 to 200 ml of water buffered at a pH of approximately 4 to 6. The cold trap is kept at a temperature of approximately 0-10° C., whereas the condenser is at a temperature ranging from approximately 10-20° C. This solution is then stirred for about 1 to 24 hours at a temperature ranging from room temperature to 45° C. Lastly, after the solution has reacted, the excess water is filtered out. Then the slurry is dried to a powder form. In this manner, a complex is formed in accordance with the present invention.

Plants are preferably exposed to a non-phytotoxic amount of the active compound. In one embodiment, approximately 0.1 gram of an encapsulated cyclopropene or derivative thereof per 50 to 500 cubic feet of atmosphere to be treated is dissolved in an aqueous solution and exposed to plants to prolong their life or inhibit their ethylene response.

The methods of the present invention involve initially the step of providing the complex of the present invention. Then the complex is dissolved to release the gaseous form of the complex. A variety of solutions may be utilized and generally encompass polar solvents, such as water, DMSO, ethanol and methanol. To expose the plant to the gaseous cyclopropene or derivative thereof, the aqueous solution is preferably positioned near the plant. Alternatively, the powder may be placed in an aerosol can containing sufficient water and 40-50 psi of compressed gas. Then, the gaseous cyclopropene may be sprayed onto the plant.

Example 3

Release of Methylcyclopropene from Cyclodextrin

To release methylcyclopropene from the cyclodextrin molecular encapsulation agent and treat plants, the first thing that should be done is to place the plants into a closed environment, preferably at elevated temperatures, preferably from 13° to 24° C. The amount of methylcyclopropene should preferably be from 100 to 500 ppb (parts per billion of methylcyclopropene in the atmosphere after release) for

crops like carnations. The amount of molecular encapsulating agent complex needed to release the proper amount of methylcyclopropene or any other compound capable of inhibiting the ethylene response in plants will depend upon the plant being treated and the specific complex formulation used. Before the active compound is released, the treating chamber is closed and the air flow arranged so that all the plants in the closed chamber will be treated. The methylcyclopropene/alpha-cyclodextrin complex is then added to water. The amount of water used should be at least 10 times the weight of the cyclodextrin and preferably 100 times the weight of the cyclodextrin. Other factors that facilitate a more complete release of the active compound capable of inhibiting the ethylene response in plants are the addition of an acidic or alkaline agent to the water so as to buffer the water to an acidic or basic pH. Additionally, the water containing the cyclodextrin complex can be heated up to 45° C. to facilitate a better release of the methylcyclopropene. The release of methylcyclopropene is faster with heating or changing pH, but in lieu of these treatments, use of a greater amount of water is sufficient to obtain a full release of the methylcyclopropene from the cyclodextrin complex. The plant treatment time is usually at least one hour, but preferably at least 6 hours unless the plants are being held at a temperature less than 15° C. in which case more time is preferred (sometimes as much as 10 hours). Once the plants are treated, the sealed chamber may be opened if desired. The methylcyclopropene is now protecting the plants because it has blocked all the available ethylene receptor sites. This treatment will protect the plants from the action of ethylene until the plant grows new unblocked ethylene receptor sites.

Example 4

Comparative Experiments

The following comparative examples demonstrate the effectiveness of the molecular encapsulation agent complexes of the present invention.

The comparative examples demonstrate the benefits of the present invention (utilizing an alpha-cyclodextrin/methylcyclopropene complex) as compared to traditional solid inert carriers, such as wood flour and molecular sieves. Specifically, these comparative examples demonstrate the amount of methylcyclopropene absorbed by traditional solid carriers as compared to that entrapped by utilizing a molecular encapsulation agent, alpha-cyclodextrin, of the present invention.

The Wood Flour Comparative Example

This experiment evaluates the differences between utilizing the complex of the present invention with a solid carrier, as proposed in U.S. Pat. No. 5,518,988. Specifically, the inventors tested the absorption amount, if any, of methylcyclopropene onto wood flour. The wood flour used was obtained from American Wood Fibers and was identified as #10010 Hardwood.

To evaluate the amount of absorption of methylcyclopropene, 0.01 grams of wood flour (previously exposed to methylcyclopropene in a buffered water solution as described below for the molecular sieve comparative example) was weighed out in a 25 ml vial, and dissolved with 5 ml of deionized water. Then, 1 ml of the headspace from the vial was injected into a gas chromatograph (a total of 20 ml of headspace was tested). In addition to testing with 0.01 grams of wood flour, 0.1 grams was also tested. Alpha-cyclodextrin was also tested under the same conditions. It was experimentally found that no methylcyclopropene attachment to the wood flour was detectable. This shows that use of a dry absorbent, such as wood flour, was not effective in absorbing methylcyclopropene.

The Molecular Sieve Comparative Example

To evaluate the differences between utilizing a molecular encapsulation agent complex of the present invention and molecular sieves, another comparative experiment was also conducted. Molecular sieves were selected for these comparison tests because they are one of the most common carriers of chemicals in the chemical industry.

Two types of molecular sieves were utilized in this comparative example, 13X and 5A. Both were obtained from the Aldrich Chemical Company in Milwaukee, Wis. Each molecular sieve was first dried at 50° C. for 30 minutes before being used. 25 grams of each were then placed in separate 250 ml Erlenmeyer flasks and cooled to -80° C. by placing then in a dry ice/acetone bath. 20 ml of methylcyclopropene (approximately 60,000 ppm) was injected into the flask and allowed to sit for 24 hours either at room temperature or at 4° C. 1 gram of molecular sieve was then weighed in a 20 ml vial, and 5 ml of deionized water was added to release the methylcyclopropene. 1 ml of the headspace from the vial was injected into a gas chromatograph to determine the concentration of methylcyclopropene adsorbed onto the molecular sieves. The following methylcyclopropene release data was obtained.

Molecular Sieve/Condition		Amount Released
13X	cooled to 4° C. for 24 hr.	15 ppm
13X	room temperature 24 hr.	15 ppm
5A	cooled to 4° C. for 24 hr.	None detected
5A	room temperature 24 hr.	None detected

The Alpha-Cyclodextrin Complex Comparative Example

The alpha-cyclodextrin/methylcyclopropene complex used in this example was made by trapping 80,000 ppm of methylcyclopropene in a 5 gallon mixing vessel with 1.3 kg of alpha-cyclodextrin in 0.575 liters of buffer solution having a pH of 4. The buffer solution was made with 0.2 M sodium acetate and 0.2 M acetic acid solutions. This is referred to as the "wet" cyclodextrin loading in the results discussed below. A "dry" cyclodextrin loading was also run. In the dry experiment, the methylcyclopropene was contacted with dry alpha-cyclodextrin, i.e., cyclodextrin that was not in an aqueous solution. In both experiments, the vessel was chilled to 4° C. and the contents mixed for 24 hours. Once the methylcyclopropene is trapped onto the cyclodextrin, the pressure fell from about 2 atmospheres to a vacuum. Nitrogen gas was then added to atmospheric pressure. The buffer solution was removed by filtering through a filtering bag within the vessel and the cyclodextrin cake was transferred to a plastic tray and allowed to air dry for 48 hours. The dry cyclodextrin with entrapped methylcyclopropene was ground with a powder grinder to a 100 mm mesh size. The complex was stored for two weeks before analysis.

To evaluate the amount of methylcyclopropene complexed or trapped by alpha-cyclodextrin, 0.01 grams of cyclodextrin (previously exposed to methylcyclopropene as described above) was weighed out in a 25 ml vial, and dissolved with 5 ml of deionized water. Then 1 ml of the headspace from the vial was injected into a gas chromatograph to determine the concentration of methylcyclopropene in the complex. The results are shown below. The methylcyclopropene was absorbed either wet or dry onto the cyclodextrin and then evaluated as described above.

Cyclodextrin loading	Amount Released
water	500-1000 ppm
dry	200-500 ppm

These results demonstrate that the 13X molecular sieve was only capable of taking up 15 ppm of methylcyclopropene. The heat of adsorption may have caused the decay of some methylcyclopropene according to the chromatographic results, but it is estimated that no more than 15 ppm could have been lost. In contrast, the results from the molecular encapsulation agent complex of the present invention demonstrate a substantially complete entrapment of the methylcyclopropene. These dramatic differences in release amounts of methylcyclopropene could not have been expected from the literature. Clearly, the molecular encapsulation agent complex of the present invention is far superior to the passive absorption to solids taught in U.S. Pat. No. 5,518,988.

What is claimed is:

1. A complex formed from a molecular encapsulation agent and a compound having the following structure



wherein n is a number from 1 to 4 and R is selected from the group consisting of hydrogen, saturated or unsaturated C1 to C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino and carboxy.

2. The complex of claim 1 wherein the molecular encapsulation agent is selected from the group consisting of a cyclodextrin, a crown ether, a polyoxyalkylene, a porphorine, a polysiloxane, a phophazene and a zeolite.

3. The complex of claim 1 wherein the compound is selected from the group consisting of cyclopropene and dimethylcyclopropene.

4. The complex of claim 1 wherein the molecular encapsulation agent is cyclodextrin.

5. The complex of claim 4 wherein the cyclodextrin is alpha-cyclodextrin.

6. A complex formed from a molecular encapsulation agent and methylcyclopropene.

7. The complex of claim 6 wherein the molecular encapsulation agent is selected from the group consisting of a cyclodextrin, a crown ether, a polyoxyalkylene, a porphorine, a polysiloxane, a phophazene and a zeolite.

8. The complex of claim 6 wherein the molecular encapsulation agent is cyclodextrin.

9. The complex of claim 8 wherein the cyclodextrin is alpha-cyclodextrin.

10. A method of delivering a compound to a plant to inhibit an ethylene response in the plant, the method comprising the step of contacting a complex formed from a molecular encapsulation agent and a compound having the following structure



wherein n is a number from 1 to 4 and R is selected from the group consisting of hydrogen, saturated or unsaturated C1 to C4 alkyl, hydroxy, halogen, C1 to C4 alkoxy, amino and carboxy, with a solvent capable of dissolving the molecular

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encapsulation agent, and thereby liberating the compound from the molecular encapsulation agent so that it can contact the plant.

11. The method of claim 10 wherein the molecular encapsulation agent is selected from the group consisting of a cyclodextrin, a crown ether, a polyoxyalkylene, a prophorine, a polysiloxane, a phophazene and a zeolite.

12. The method of claim 10 wherein the compound is selected from the group consisting of cyclopropene and dimethylcyclopropene.

13. The method of claim 10 wherein the molecular encapsulation agent is cyclodextrin.

14. The method of claim 13 wherein the cyclodextrin is alpha-cyclodextrin.

15. The method of claim 10 wherein the solvent comprises water.

16. The method of claim 15 wherein the water additionally comprises an acidic or alkaline agent.

17. The method of claim 10 further comprising bubbling a gas through the solvent while it is in contact with the complex.

18. The method of claim 10 further comprising applying heat to the solvent either before it contacts the complex or during that contact.

19. A method of delivering methylcyclopropene to a plant in order to inhibit an ethylene response in the plant, the

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method comprising the step of contacting a complex formed between a molecular encapsulation agent and methylcyclopropene with a solvent capable of dissolving the molecular encapsulation agent, and thereby liberating the methylcyclopropene from the molecular encapsulation agent so that it can contact the plant.

20. The method of claim 19 wherein the molecular encapsulation agent is selected from the group consisting of a cyclodextrin, a crown ether, a polyoxyalkylene, a prophorine, a polysiloxane, a phophazene and a zeolite.

21. The method of claim 19 wherein the molecular encapsulation agent is cyclodextrin.

22. The method of claim 21 wherein the cyclodextrin is alpha-cyclodextrin.

23. The method of claim 19 wherein the solvent comprises water.

24. The method of claim 23 wherein the water additionally comprises an acidic or alkaline agent.

25. The method of claim 19 further comprising bubbling a gas through the solvent while it is in contact with the complex.

26. The method of claim 19 further comprising applying heat to the solvent either before it contacts the complex or during that contact.

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Release of 1-Methylcyclopropene from Heat-Pressed Polymer Films

J.H. HOTCHKISS, C.B. WATKINS, AND D.G. SANCHEZ

ABSTRACT: Gaseous 1-methylcyclopropene (1-MCP) is an inhibitor of ethylene perception that is being used extensively for apples and ornamental products, and under intensive investigation for its potential benefits for other fruits and vegetables. 1-MCP is currently used in closed environments that maintain stable concentrations for several hours in order to be effective. However, food packaging materials that release 1-MCP at a predictable rate into the package headspace might be useful for application in inhibiting the deleterious effects of ethylene in the postharvest packaging and storage of some horticultural products. A 1-MCP/ α -cyclodextrin (1-MCP-cd) complex was incorporated into several common packaging films by heat-pressing (dry-blend, lamination) and solution-casting methods. The release of 1-MCP from the films was quantified by gas chromatography with respect to time, loading of 1-MCP, temperature, relative humidity (RH), type of film, and film-forming method. Release of 1-MCP was rapid and high in films held at RH $\geq$ 75%. The rate of release was slow during the 1st 12 h and then increased during the next 24 to 36 h. Higher temperatures resulted in higher and faster release. A loading of 8 mg of 1-MCP-cd per 140 mg of polymer was found to be optimal. Pressing 1-MCP-cd containing films above 100 °C reduced the amount of 1-MCP remaining in the film. Incorporation into LDPE resulted in a higher and faster release than from PS, PVC, and PP polymers. 1-MCP release from a film matrix appears to be within the acceptable range for produce packaging applications.

Keywords: active, film, packaging, polymer, 1-methylcyclopropene (1-MCP)

Introduction

Ethylene is a plant growth regulator that affects many aspects of plant growth and development and from a postharvest perspective can have major effects on ripening and senescence (Saltveit 1999). These effects can be both positive and negative (Saltveit 1999), but typically control of ethylene production by technologies such as low temperature and modified atmospheres, as well as avoidance, ventilation, and chemical destruction of ethylene, is used by industry to maintain the quality of horticultural products (Watkins 2002). Recently, a new technology based on 1-methylcyclopropene (1-MCP), an inhibitor of ethylene binding (Sisler and Serek 2003), has been developed and approved for food use (EPA 2002). 1-MCP has been registered for use on a range of horticultural products, the fruit or vegetable being specific to each country (Watkins 2007). 1-MCP is already used extensively on apples and some ornamental products and is the subject of intense study around the world as a means of maintaining quality for fruits and vegetables (Blankenship and Dole 2003; Watkins 2006).

Commercial application of 1-MCP to horticultural products is based on release of gaseous 1-MCP from a 1-MCP/ α -cyclodextrin complex in either powder or tablet form after addition of water or base solution. 1-MCP application is carried out in sealed rooms to ensure exposure of the crop to the chemical for several hours. Other strategies for 1-MCP application could include the combining of 1-MCP with an active packaging system. The patent literature (Kostansek 2002) suggests that low concentrations of cyclopropenes can be released from packaging materials that incorporate the cyclopropene. However, the use of 1-MCP in combination with mod-

ified atmospheres (MA) and/or packaging has been limited. The use of MA for prepackaging 1-MCP-treated bananas and mangoes has been reported (Jiang and others 1999; Jiang and Joyce 2000). Another possible use of 1-MCP in packaging could be for fresh-cut or minimally processed products as ethylene production is increased in many fresh-cut fruits and vegetables (Rolle and Chism 1987). Preventing accumulation of ethylene around fresh-cut products reduced the rate of softening in kiwifruits and bananas and of chlorophyll loss in spinach leaves, but not in broccoli (Abe and Watada 1991), but exacerbated chilling injury of tomato (Hong and Gross 2000). 1-MCP extended the storage life of fresh-cut lettuce (Wills and others 2002; Tay and Perera 2004), pineapple (Budu and Joyce 2003), and papaya (Ergun and others 2006), although 1-MCP does not always improve storability of fresh-cut products, for example, apple (Bai and others 2004) and banana (Vilas-Boas and Kader 2006). Where beneficial effects of 1-MCP have been obtained, these have sometimes been via its effects on maintaining better quality of the preprocessed product (Calderon-Lopez and others 2005; Ergun and others 2006). Lee and others (2006) have reported on the release of 1-MCP from package sachets but no data on the release from films are, to our knowledge, available.

Our objective was to investigate the release of 1-MCP from polymer films into which 1-MCP-cd complex had been incorporated. The 1-MCP-cd complex was incorporated into several common packaging films by heat-pressing (dry-blend) and solution-casting methods, and the release of 1-MCP from the films was quantified with respect to time, loading of 1-MCP, temperature, relative humidity (RH), type of film, and film forming method.

Materials and Methods

Reagents (or active compound)

Technical grade SmartFresh™ containing 1-MCP/ α -cyclodextrin complex (1-MCP-cd; 4.4% active ingredient) was obtained from

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AgroFresh Inc. (Rohm and Haas Co., Springhouse, Pa., U.S.A.). Technical grade consists of a 1:1 complex of 1-MCP in α -cyclodextrin (cd), uncomplexed cd, and approximately 6% to 8% water of crystallization. Gaseous 1-MCP is displaced from the cd by water, releasing it to the atmosphere. The SmartFresh powder was held under desiccation to prevent 1-MCP release until use. All polymer resins were purchased (Scientific Polymer Products Inc., Ontario, N.Y., U.S.A.) and were additive free.

Film formation

Cast films were obtained by mixing 10 mg 1-MCP-cd with 1 g of polymer resin which had been dissolved in an appropriate solvent. The mixture was poured onto a 15-cm glass plate and the solvent evaporated overnight in the dark at room temperature. After drying, the films were peeled off the plate, and stored until use as described below.

Heat-pressed films were prepared by thoroughly dry blending powdered polymer resins (LDPE, PVC, PP, PS, and EVOH) and technical grade (that is, SmartFresh) 1-MCP-cd (10 mg 1-MCP-cd powder [440 μ g of 1-MCP] per gram of polymer) in powdered form, then heat pressing the dry blend between 2 sheets of Mylar set between the 2 preheated plates of a hot press (Model 341-20, Loomis Engineering & MFG. Co., Caldwell, N.J., U.S.A.). The temperature of the

plates was stabilized at the desired pressing temperature (115 to 185 °C). The sheets were pressed and heated for 1 min at 210000 kPa. The films (approximately 102 μ m of thickness) were peeled from the Mylar sheets and stored desiccated at ambient temperature until testing (1 to 2 d).

The release of 1-MCP from the compounded films was determined by placing the entire film sample in a 130-mL glass jar (Ball Corp., Muncie, Ind., U.S.A., product code 14400-80400) along with a metal clip to hold the film and a 2.0-mL vial containing the appropriate saturated salt solution (Wexler and Hasegawa 1954) or distilled water to provide the desired RH. The jars were sealed with metal lids fitted with rubber septa (Wheaton, Millville, N.J., U.S.A.) and further secured with silicone rubber sealant (General Electric Co., Waterford, N.Y., U.S.A.) which served as a sampling port. The amount of 1-MCP released from the film or 1-MCP-cd control over 120 h was determined by periodically analyzing the headspace in the jars for 1-MCP concentration. Headspace (0.5 mL) was withdrawn by gas-tight glass syringe (Model nr 81220, Hamilton Co., Reno, Nev., U.S.A.) and analyzed by gas chromatography with flame ionization detector (GC-FID). GC-FID conditions were Varian 6000 GC (Varian Inc., Walnut Creek, Calif., U.S.A.); CP-PoraPlot Q-HT fused silica capillary column 10 m $\times$ 0.53 mm with a 10- μ m film;

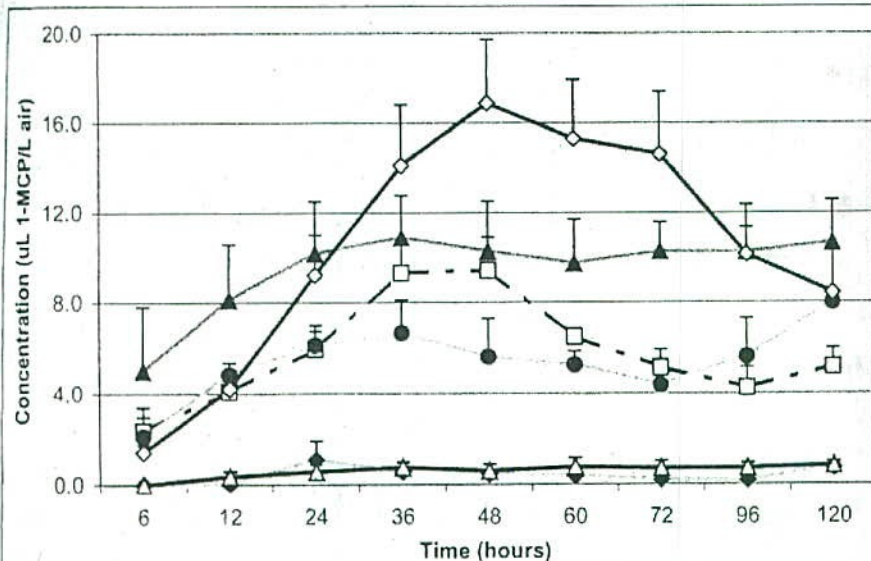


Figure 1—Headspace concentration of 1-MCP released from different polymer films containing 1-MCP-cd and pressed at different temperatures: LDPE, 115 °C (Δ), PS 135 °C (□), PVC, 142 °C (●), PP, 165 °C (◇), LDPE 185 °C (◇), and 1-MCP-cd control (◇). All films and the 1-MCP control were held at 22 °C and 100% RH. Vertical bars represent 1 standard deviation (n = 3).

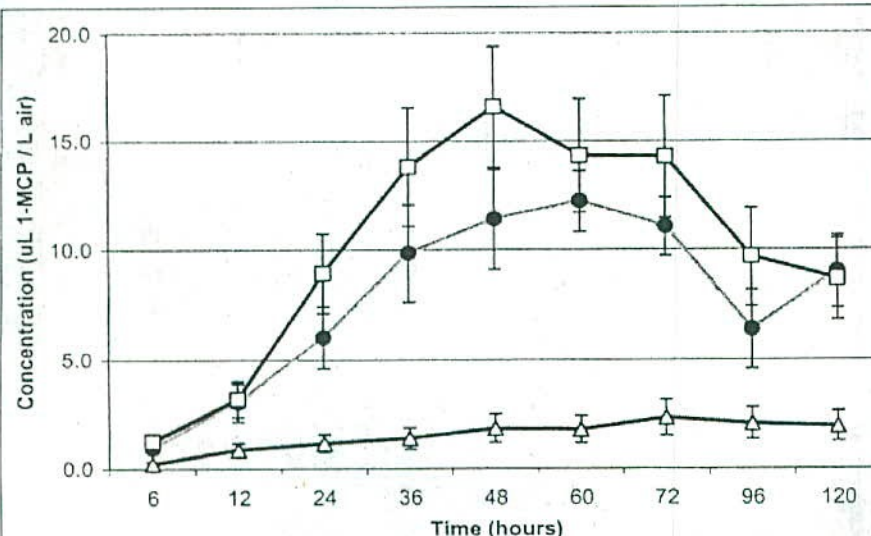


Figure 2—Headspace concentration of 1-MCP released from films containing 1-MCP-cd heat-pressed at 115 °C (●), 185 °C (Δ), 1-MCP control (□). All films and 1-MCP control were held at 22 °C and 100% RH. Vertical bars represent $\pm$ 1 standard deviation.

(Catalog nr 7558, Chrompack, The Netherlands) column temperature was 140 °C/min, increased to 200 °C at 30 °C/min, held at 200 °C for 3 min; carrier gas 2.5 mL/min N₂. 1-MCP concentration was determined against a 48.8- μ L/L isobutylene in air external standard (Scott Specialty Gases, South Plainfield, N.J., U.S.A.). This assumes that the response factor for 1-MCP and isobutylene is unity. A Varian 4290 Integrator (Varian Inc., Walnut Creek, Calif., U.S.A.) was used to quantify the 1-MCP and isobutylene peaks. Three separate jars were used for each measurement.

Permeability measurements

A permeation apparatus as described by Al-Ati and others (2003) was used to determine the permeation rates for 1-MCP through LDPE films.

Statistical analysis

Minitab Release 14 (Minitab Inc., State College, Pa., U.S.A.) was used to test significance using the general linear model to determine the treatment and interaction effects and Tukey's test for comparing the levels. Three replicates per treatment and 10 slices per replicate were used in all experiments. Data were tested for multiple comparisons by analysis of variance (ANOVA) with least significant difference (LSD) between averages determined at the 5% level.

Results and Discussion

Only trace amounts of 1-MCP were released from any cast film. For example, the headspace concentration of 1-MCP released from cast PS and LDPE films reached 0.34 and 0.44 μ L/L after 20 h, while the release of 1-MCP from 1-MCP-cd that had not been incorporated into film was 10 to 15 μ L/L. We believe that 1-MCP was most likely lost during the solvent evaporation processes. This suggests that solvent casting may not be suitable for forming films that incorporate 1-MCP-cd. Therefore, all subsequent work was conducted with heat-pressed films.

The headspace concentrations of 1-MCP released from heat-pressed LDPE, PS, and PVC films were 10.8, 9.3, and 6.7 μ L/L with maximum concentration being reached in 36 to 48 h (Figure 1). The control (1-MCP-cd alone) followed a similar time to maximum concentration but the absolute concentration was 17 μ L/L. PP film pressed at 165 °C and LDPE film pressed at 185 °C resulted in only trace 1-MCP concentrations (<1 μ L/L). No detectable 1-MCP was released from heat-pressed EVOH films. These low release amounts were likely due to the high pressing temperature (185 °C), resulting in volatilization of the 1-MCP from the matrix. This is supported by the observation that LDPE films that were pressed at 185 °C had a much lower release than LDPE films pressed at 115 °C, suggesting that polymer processing temperature would have a large effect on the incorporation of 1-MCP.

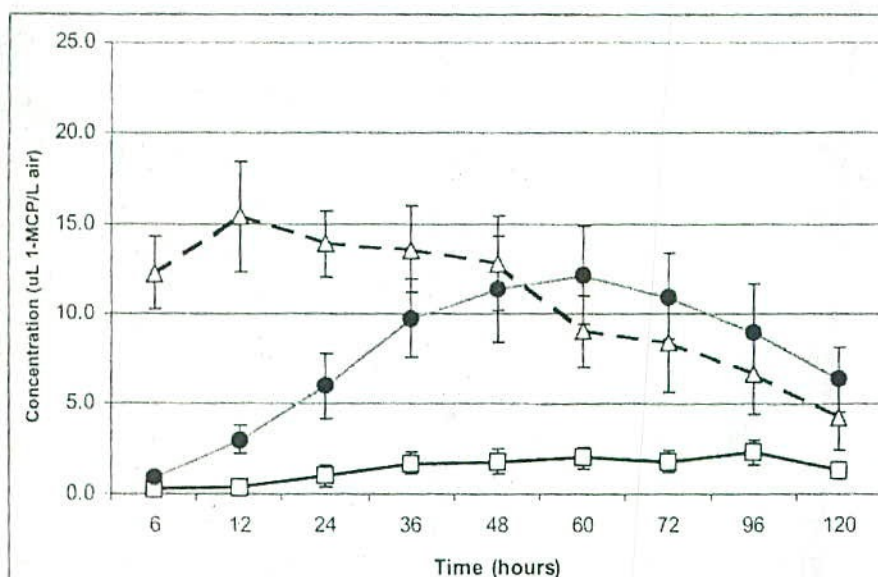


Figure 3—Headspace concentration of 1-MCP from heat-pressed LDPE films containing 1-MCP-cd and held at 56 °C (Δ), 22 °C (●), and 5 °C (□) for 6 to 120 h at 100% RH. Vertical bars represent ± 1 standard deviation ($n = 3$).

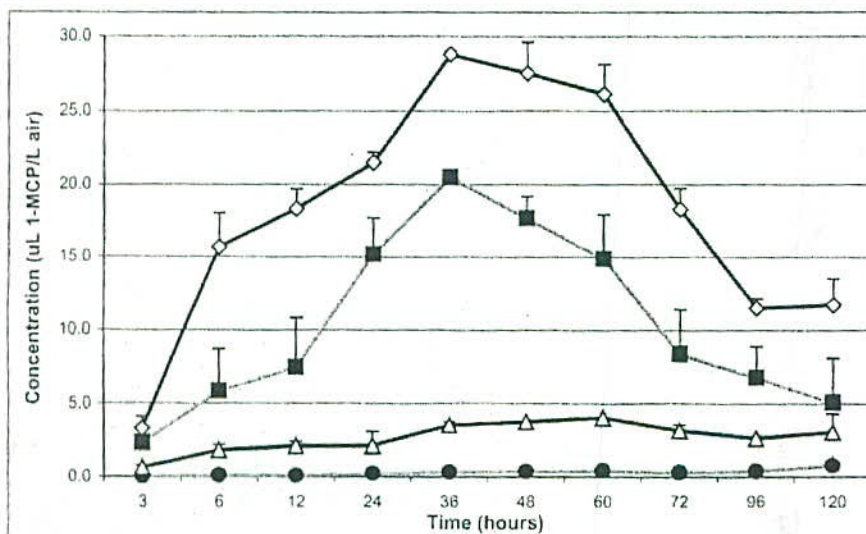


Figure 4—Headspace concentration of 1-MCP from 1-MCP-cd at 24 °C and 100% RH (◇), 75% RH (■), 52% RH (Δ), and 32% RH (●). Vertical bars represent 1 standard deviation.

polymer films (Figure 2). In all cases, including the controls, the concentration of 1-MCP in the jars decreased slightly after reaching a maximum. This was most likely due to absorption of the 1-MCP by the silicone rubber septum and/or the rubber sealing compound in the jar lids.

The optimum time and pressure for forming films incorporating 1-MCP-cd was 1 to 2 min at a pressure of 210000 kPa and a 5.5% loading 1-MCP-cd (w/w) and a heat press temperature of 115 °C, which is near the lower melt limit for LDPE (data not shown). This short-time and low-temperature process was thus used for further studies since it resulted in a higher release of 1-MCP. Adding 57 mg of 1-MCP-cd/g of LDPE (5.5% loading) was found to result in a suitable film with minimal loss of 1-MCP after a series of loadings were analyzed (1%, 3%, 10%, 15%, and 20%).

Increasing the temperature in which the jars containing films were held increased the rate of 1-MCP release from both free 1-MCP-cd and film containing 1-MCP-cd (Figure 3). Plotting the logarithm of headspace concentration against the reciprocal of temperature gave a straight line, which suggested that release followed Arrhenius behavior (not shown). The release from films was rapid and gave the highest concentration when held at 56 °C compared with lower temperatures. In contrast, 1-MCP-incorporated LDPE films held at 5 °C gave the lowest and slowest releases of 1-MCP (2.3 µL/L). In practice, this temperature release sensitivity should be taken into account.

When temperature was held constant and RH varied, the release of 1-MCP increased as the RH increased (Figure 4). At 36 h, 1-MCP release was 0.4 µL/L at 32% RH, 3.5 µL/L at 52% RH, 20.5 µL/L at 75% RH, and 28.8 µL/L at 100% RH, respectively.

The permeability of 1-MCP through LDPE at 100% RH resulted in a characteristic Fickian permeation and time lag curve (Figure 5). The permeability, solubility, and diffusivity coefficients for 1-MCP in LDPE were calculated as $D = 2.23 \times 10^{-9} \text{ cm}^2/\text{s}$, $S = 60.01 \text{ cm}^3 (\text{STP})/(\text{cm}^3 \cdot \text{atm})$, and $P = 13.4 \times 10^{-8} (\text{cm}^3 (\text{STP}) \cdot \text{cm})/(\text{cm}^2 \cdot \text{atm} \cdot \text{s})$. 1-MCP appears to readily pass through the polymer films tested. The diffusivity value of 1-MCP was lower than that of CO_2 and O_2 as was expected since 1-MCP is a larger molecule.

The above experiments were all conducted in vessels containing distilled water (that is, RH = 100%). In order to determine if cut fruits would provide sufficient moisture to cause release of 1-MCP, a $1.5 \times 1.5 \times 1.5 \text{ cm}$ piece of fresh-cut apple was used in lieu of water to generate humidity within the container. The control for this experiment consisted of a similar sample of a 1-MCP-incorporated film that was placed inside the test system without any RH source. An additional control consisted of a 1-MCP-cd containing film and a 2-mL vial of distilled water to generate 100% RH. The humidity provided by the fresh-cut apple cube was sufficient to liberate the 1-MCP from the film (Figure 6). The release from the dry 1-MCP-cd was insignificant (data not plotted), while the release of 1-MCP from a 100% RH environment occurred at a faster rate than the apple

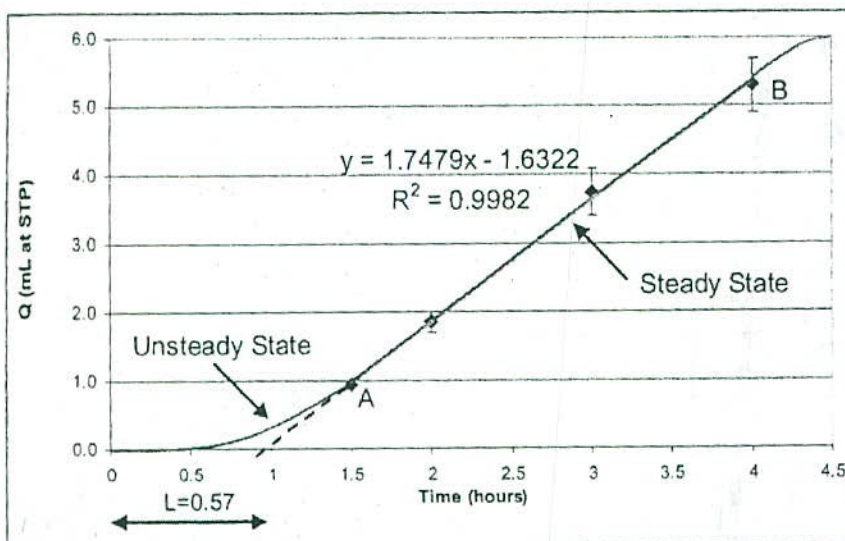


Figure 5—Permeation and time lag curve, where Q is the amount of penetrant (1-MCP), as a function of time for LDPE films. Extrapolation of the steady-state line AB to the time axis gives the time lag L as intercept, which along with the slope (Q/T) allowed us to calculate the permeability, solubility, and diffusivity values of 1-MCP in LDPE: $D = 2.23 \times 10^{-9} \text{ cm}^2/\text{s}$, $S = 60.01 \text{ cm}^3 (\text{STP})/(\text{cm}^3 \cdot \text{atm})$, and $P = 13.4 \times 10^{-8} (\text{cm}^3 (\text{STP}) \cdot \text{cm})/(\text{cm}^2 \cdot \text{atm} \cdot \text{s})$. Vertical bars represent ± 1 standard deviation.

$$S = 1.53 \times 10^{-6} \text{ g}/\text{cm}^3 \cdot P_a$$

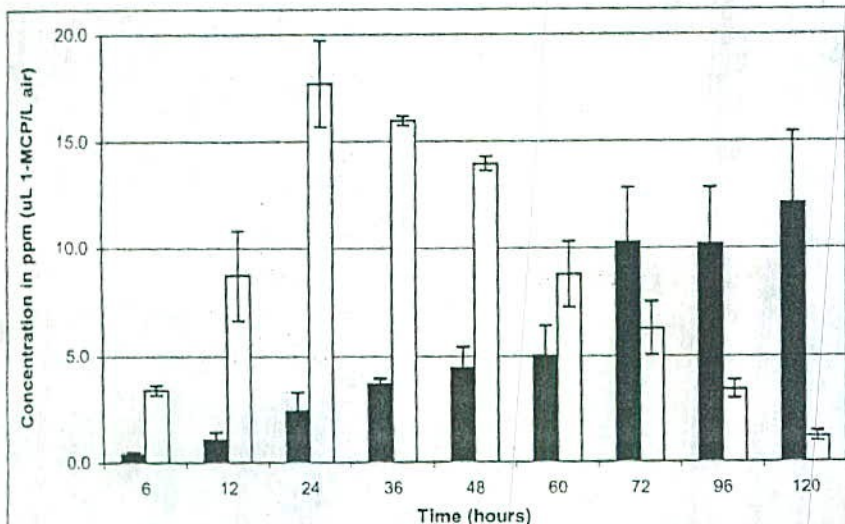


Figure 6—Headspace concentration of 1-MCP as a function of storage time: via moisture activated release of fresh-cut apple slices (■) compared with the 1-MCP-cd control (□). All films and 1-MCP-cd control were held at 22 °C. Vertical bars represent ± 1 standard deviation.

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slice. These data indicate that 1-MCP was released from the LDPE film by the humidity originating from the apple piece. The apple tissue will absorb some of 1-MCP, which might be a factor in the final concentration achieved.

Conclusion

Polymer processing method and temperature strongly affected 1-MCP release, with low-temperature heat-pressed films having the highest release rates. The rate of release of 1-MCP, which occurred readily above 75% RH, was slow during the 1st 12 h but then increased during the next 24 to 36 h. A loading of 5.5% (8 mg of 1-MCP-cd per 140 mg of LDPE) was found to be the optimum for the creation of uniform polymer films. Higher holding temperatures result in a higher and faster release of the active compound. Taken as a whole, these results suggest that 1MCP-cd could be incorporated into a packaging film which would subsequently release 1-MCP during the storage of fresh-cut produce.

Additional work to control the release characteristics of 1-MCP from the packaging film is needed to further develop this concept. Evaluation of a broader range of films and layering structures should be undertaken. In addition, commercial application will require higher quality films and therefore refinement of the pressing process to optimize pressing time, loading, and temperature. Also, the effects of this concept on specific produce items will need to be studied. A multilayered structure in which the outer layer is a high barrier to 1-MCP could be used to prevent loss to the general environment. This work also indicates that loss of 1-MCP during film storage could be prevented by ensuring that the films are held in low-humidity environments or packaged in high barrier films until use.

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Kinetic Study of Thermally Stimulated Dissociation of Inclusion Complex of 1-Methylcyclopropene with α -Cyclodextrin by Thermal Analysis

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The thermally stimulated dissociation of the inclusion complex of 1-methylcyclopropene (1-MCP) with α -cyclodextrin (α -CD) in solid state was studied by means of thermogravimetry (TG) and differential scanning calorimetry (DSC). The mass loss of 1-MCP/ α -CD inclusion complex occurs in four separated phases with the thermal dissociation of the inclusion complex and release of 1-MCP taking place in the second phase between 90 and 230 °C. The kinetic parameters of the dissociation reaction (the apparent activation energy of dissociation, E_D , the reaction order of thermal dissociation, n , and the pre-exponential factor, k_0) were evaluated. The dissociation reaction was satisfactorily described by the unimolecular decay law, where the reaction order, $n = 1$. The effect of the molar ratio of 1-MCP to α -CD (inclusion ratio) in the inclusion complex on the temperature dependence of the dissociation reaction was also studied. The E_D decreased with increasing inclusion ratio indicating higher complex stability at lower inclusion ratios. The extrapolation of the E_D of the inclusion complexes with different inclusion ratios to 1 mol 1-MCP/mol α -CD yielded the "true" E_D of 20.9 ± 2.8 and 18.1 ± 0.2 kJ/mol for TG and DSC, respectively. The "true" $\ln k_{0TG}$ and the "true" $\ln k_{0DSC}$ were also determined by extrapolation, yielding values of $+4.5 \pm 1.0$ and -0.3 ± 0.3 , respectively.

Introduction

Olefin compounds that chemically inhibit ethylene perception in plants and thereby counteract ethylene in inducing physiological responses namely senescence, abscission, color change, and ripening,¹ have been attracting a great deal of attention in the horticultural field. As an ethylene inhibitor, 1-methylcyclopropene (1-MCP) is effective and commercially available, and thus of considerable interest.² Since discovery, 1-MCP has resulted in an explosion of research on its effects on fruits, vegetables, and ornamental crops.^{3,4} Food use registration for 1-MCP has been obtained in countries like Australia, Austria, Brazil, Canada, Israel, The Netherlands, New Zealand, South Africa, the U.K., the U.S.A., etc.,¹ and registration in Japan is still underway. The commercialization of 1-MCP was followed by rapid adoption by many apple industries around the world.³ 1-MCP effectively inhibits ethylene perception at extremely low concentrations over an extended period of time² via a nontoxic mode of action.⁵ For the purpose of speculating its potential applications, considerably extensive studies that cover a wide variety of produce and ornamentals including apple, apricot, banana, mango, pear, broccoli, lettuce, carnation, rose, etc., have consequently been carried out, in which details of the action, application, and effects of 1-MCP are reported.^{6–21}

In addition to the aforementioned applications, 1-MCP is also a valuable substance in fundamental scientific researches, especially as a tool that helps scientists make major advances in understanding the role of ethylene in plants and gain insight into the fundamental processes that are involved in ripening and senescence of fresh produce.³ In a study by Nakatsuka et al., 1-MCP was used to examine the regulation of ethylene synthesis in tomato fruit and their findings suggested that a strong positive feedback regulation was involved in ethylene

biosynthesis at the gene transcriptional level.²² Mwaniki et al. also utilized 1-MCP for elucidation of the role of ethylene in the regulation of genes encoding β -galactosidase and hence the softening process in "La France" pear (*Pyrus communis* L.).²³ Both ethylene and 1-MCP were used by Mainardi et al. to artificially induce or delay the ripening of banana (*Musa accuminata* AAA cv. Nanicão) so as to investigate the gene expression and activity profile of α -1,4-glucan-phosphorylase during the ripening process.²⁴

Cyclodextrins (CD) are water-soluble cyclic oligosaccharides made up of 6, 7, and 8 α -1,4-linked glucopyranose units designated respectively as α -, β -, and γ -cyclodextrin. The truncated molecular structure of CD gives rise to a unique torus-shaped cavity which is capable of including a great variety of substances at molecular level to form a stable inclusion complex (host–guest complex) in both solid and aqueous phases. The so-called guest molecules encompass substances of solid (menthol,^{25,26} imidazole derivatives²⁷), liquid (flavors^{28,29}), and gaseous states (1-MCP,³⁰ CO₂^{31,32}).

1-MCP is currently being commercialized as a water-soluble powder that comprises its inclusion complex with α -CD, the form in which it is stable under dry condition with greatly diminished explosiveness.³³ With the solid-state inclusion complex being mostly the form in which 1-MCP is handled at present, studying the solid-state thermal dissociation kinetics would provide an insight into the heat stability of the inclusion complex. Instead of being limited to batch application in closed environments, a better understanding of the heat stability of the inclusion complex may help develop new ways of application especially those that require some pretreatment with heat. For instance, in the study on 1-MCP release from packaging films incorporated with 1-MCP/ α -CD inclusion complex by Hotchkiss et al.,³⁴ the data on the heat stability of the inclusion complex would be a useful piece of information particularly for the preparation of the films by heat-pressing.

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Besides analytical methods such as IR, NMR, UV spectroscopy, X-ray diffractometry, and chromatography, thermal analysis has also been applied in the characterization of CD and their inclusion complexes.^{35–40} A review on the applications of thermal methods to CD and their inclusion complexes was published by Giordano et al.⁴¹ In most of the reported works, however, thermoanalytical data were meant only for qualitative analyses. There are rather few papers being published on the kinetic study of either the inclusion or the dissociation reaction of CD inclusion complexes based on the thermoanalytical data. Despite the fact that it is of utter importance to study the dissociation of 1-MCP/ α -CD inclusion complex in response to heat to evaluate the heat stability of the inclusion complex, the kinetics of thermal dissociation of the inclusion complex still remains unexplored to the best of our knowledge. We devoted this work to studying the thermally stimulated dissociation kinetics of 1-MCP/ α -CD inclusion complex in solid state. The dissociation reaction was investigated under nonisothermal conditions by thermogravimetry (TG) and differential scanning calorimetry (DSC). Furthermore, the molar ratio of 1-MCP to α -CD (inclusion ratio) of the inclusion complex was varied for investigation of the effect on the dissociation reaction. Our study leads to the establishment of kinetics parameters namely the “true” apparent activation energy of thermal dissociation, E_D , the dissociation reaction order, n , and the “true” pre-exponential factor, k_0 that account for the dissociation reaction. The “true” E_D , the “true” k_{0TG} , and the “true” k_{0DSC} were defined as the respective kinetic parameters estimated at the inclusion ratio of 1 mol 1-MCP/mol α -CD.

Materials and Methods

Materials. α -CD ($(C_6H_{10}O_5)_6$, $M_w = 973$, purity $\geq 99\%$) was obtained from Ensui Sugar Refining Co., Ltd. (Tokyo, Japan). The α -CD crystals were dried in-vacuo at 90 °C for 24 h before use. All the chemicals used were of reagent grade unless otherwise indicated and the chemicals were used without additional purification. 3-Chloro-2-methylpropene (98%) and lithium diisopropylamide (30 wt % suspension in mineral oil) were purchased from Sigma-Aldrich Japan K. K. (Tokyo, Japan). Nitrogen was purchased from Iwatani Industrial Gases Corporation (Osaka, Japan) in gas cylinders. Distilled water was used throughout the entire experiment.

1-MCP Synthesis. 1-MCP was synthesized according to the method reported by Sisler and Serek⁴² with some modifications as has been described in detail in our previous study.³⁰ Briefly, 2.4 mL of 3-chloro-2-methylpropene (98%) was reacted with 21.42 g of lithium diisopropylamide (30 wt % suspension in mineral oil) and 1-MCP was formed as lithium salt suspended in mineral oil. After reaction, high vacuum was pulled on the suspension liquid to eliminate volatile impurities particularly the remaining 3-chloro-2-methylpropene. The collected suspension liquid was then stored at -25 °C until use. During the encapsulation procedure, the frozen suspension liquid was first thawed and then reacted with distilled water to produce 1-MCP gas, which subsequently complexes with α -CD.

Preparation of 1-MCP/ α -CD Inclusion Complex. As has been previously reported,³⁰ inclusion complexation of 1-MCP with α -CD was carried out in a hermetic agitated vessel. An α -CD solution of 50 mM at 20 °C was prepared on the basis of 100 g of distilled water and filled into a 500 mL SCHOTT DURAN laboratory glass bottle (DURAN Produktions GmbH and Co. KG, Mainz, Germany) with a modified screw cap. This bottle, designated as the encapsulation vessel, was immersed in a 20

°C water bath for temperature equilibration prior to the encapsulation process.

Meanwhile, vacuum was pulled to about 2.7 kPa in another SCHOTT DURAN 500 mL laboratory glass bottle, designated as the reaction vessel, which contained 100 g of distilled water using the PTFE diaphragm vacuum pump V-700 connected to the vacuum controller V-850 (BÜCHI Labortechnik AG, Flawil, Switzerland). Roughly 3 g of previously thawed suspension liquid of lithium salt of 1-MCP was injected into the reaction vessel through a rubber septum (Shimadzu Corp., Kyoto, Japan) fitted on the screw cap. Agitation was performed for about 15 min to promote complete reaction and evaporation of 1-MCP gas into the head space.

Next, vacuum was also pulled to about 2.7 kPa in the encapsulation vessel containing temperature-equilibrated α -CD solution. Transfer of 1-MCP from the reaction vessel to the encapsulation vessel was carried out by connecting the two vessels to one another with a Teflon tube of 6 mm i.d. through the BVLM 20-0808 bulkhead union elbows (Pisco USA, Inc.) installed on the screw caps of each vessel. Nitrogen was filled into the reaction vessel to create atmospheric pressure to promote 1-MCP transfer until the pressure of both vessels reached the atmospheric value. Encapsulation was carried out at 20 °C and promoted by agitation of the α -CD solution at an agitation rate of 200 rpm for 6 h. At the end of the encapsulation process, the 1-MCP/ α -CD inclusion complex was collected as a precipitate by centrifugation (3000 rpm, 15 min). The recovered wet precipitate was then dried in-vacuo at ambient temperature for 24 h.

Preparation of Inclusion Complex Varying in Inclusion Ratio. The inclusion ratio of 1-MCP/ α -CD inclusion complex is defined as the molar ratio of 1-MCP to α -CD in the inclusion complex itself. The inclusion ratio of the inclusion complex was varied to investigate the effect of inclusion ratio on the thermal dissociation of 1-MCP from α -CD. The inclusion complex powder collected after vacuum drying was subjected to heat treatment at 120 °C in the Advantec VR-320 vacuum drying oven (Advantec Toyo Kaisha, Ltd., Tokyo, Japan).

With treatment time, the inclusion ratio was reduced progressively from 0.72 to 0.66, 0.53, and 0.39 mol 1-MCP/mol α -CD.

Thermogravimetry (TG). Mass loss in 1-MCP/ α -CD inclusion complex as a function of temperature was recorded on the EXSTAR 6000 TG/DTA (TG/DTA 6200, SII Nano Technology Inc., Tokyo, Japan) equipped with the Muse Measurement software, version 3.7 (SII Nano Technology Inc.). The equipment was calibrated prior to measurement using a 20 mg weight. Measurements were performed by nonisothermal method on samples of masses within 5 ± 0.5 mg in open aluminum pans. The samples were analyzed at three different heating rates of 5, 7, and 10 °C/min from 25 to 300 °C under a nitrogen flow at 300 mL/min. An open empty aluminum pan was used as the reference. Triplicate measurements were made for each treatment. The data were analyzed using the Muse Measurement software, version 3.7.

Differential Scanning Calorimetry (DSC). A preliminary study was carried out to determine the thermal lag of the calorimeter cell based on the melting of high purity indium. An arbitrary weight of indium chip was sandwiched in the middle between two layers of 2.5 mg of inclusion complex, in the way that it was insulated by the inclusion complex from the aluminum pan wall, so as to obtain reliable data on the thermal lag of the system. The samples were subjected to DSC measurements at heating rates of 2, 5, 10, and 20 °C/min within

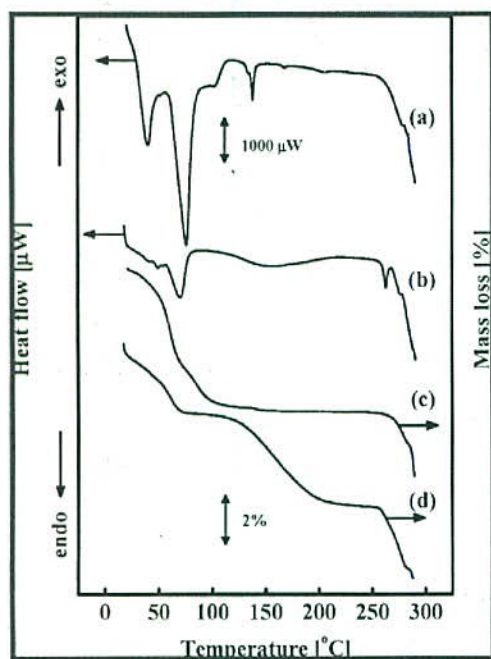


Figure 1. Typical DSC and TG curves of 5 mg samples of the uncomplexed α -CD (a and c) and the 1-MCP/ α -CD inclusion complex (b and d), respectively. The thermal analysis was carried out at 5 °C/min.

25–300 °C. The thermal lag was determined as the shift of the endothermic melting peak of indium from the reference value of 156.6 °C as a result of the insulation. The maximum thermal lag was 2.1 °C recorded at 20 °C/min. Because the highest heating rate employed in this study was 10 °C/min, which revealed a lag of only 1.2 °C, the thermal lag was regarded as negligible.

Heat flow in the inclusion complex during temperature increment was measured using the EXSTAR 6000 DSC (DSC 6220, SII Nano Technology Inc.) equipped with the Muse Measurement software, version 3.7 (SII Nano Technology Inc.). Temperature and sensitivity calibration of the equipment was performed prior to measurement using high purity indium (Mp, 156.6 °C; ΔH , 28.59 mJ/mg) and stannum (Mp, 232.0 °C; ΔH , 60.62 mJ/mg). All the measurement conditions—the range of sample size, heating rate, and temperature, and the reference material—are identical to that applied in the TG analysis, except for the nitrogen flow rate, which was set at 30 mL/min. The aluminum pans were also left open to obtain results that are comparable with that from the TG analysis. Measurements were made in triplicate for each treatment and the data were analyzed using the Muse Measurement software, version 3.7.

Results and Discussion

Thermal Characterization of 1-MCP/ α -CD Inclusion Complex. Figure 1 represents the typical DSC and TG curves of 5 mg samples of the uncomplexed α -CD (a and c) and the 1-MCP/ α -CD inclusion complex (b and d), respectively, recorded at the heating rate of 5 °C/min. CD are known to crystallize as hydrates, with water molecules in part included in the cavity of the cyclic macromolecule and in part localized in the interstices between macromolecules within the crystal lattice.⁴³ The uncomplexed α -CD demonstrated a wide dehydration phase, which occurred from 20 to 120 °C as endothermic processes with two sharp peaks at 40.4 and 76.2 °C, and a shallow peak at 102.7 °C in the DSC curve (Figure 1a). The correspondence of the endothermic peaks with dehydration has earlier been reported

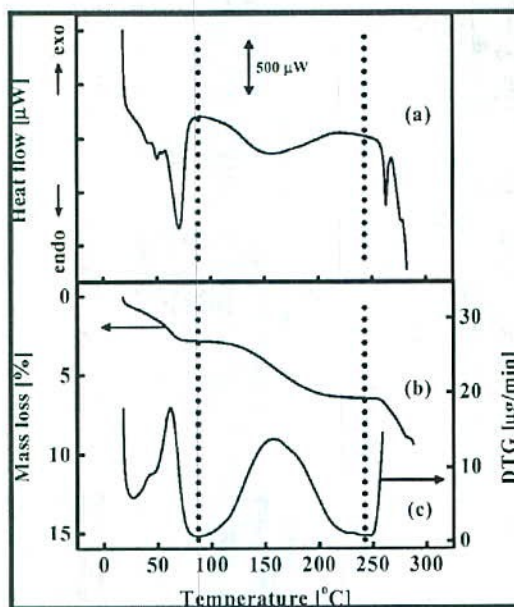


Figure 2. Typical DSC (a), TG (b), and DTG (c) curves of 5 mg 1-MCP/ α -CD inclusion complex recorded at 5 °C/min. The dashed lines define the temperature region of thermal dissociation.

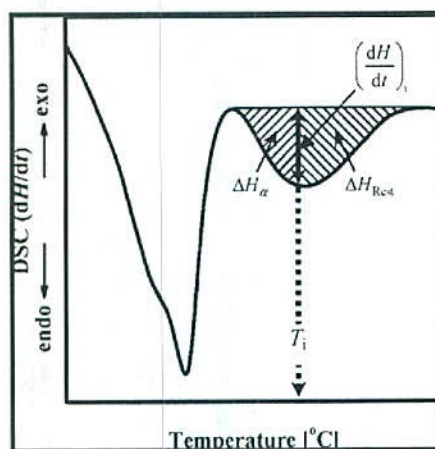


Figure 3. Definition of ΔH_a , ΔH_{Rest} , and $(dH/dt)_i$ used for determination of kinetic parameters from the DSC curve.

by researchers who have studied on the dehydration of α -CD of different polymorphs by thermal analysis.^{44–46} Bettinetti et al.⁴³ observed a two-step dehydration DSC patterns with a slight shoulder after the second peak for one of their α -CD samples, which they claimed to be characteristic for form I α -CD hexahydrate polymorph. Meanwhile, Bettinetti et al.⁴⁶ reported a fairly similar dehydration profile of form I α -CD hexahydrate to ours, which was composed of two sharp endothermic peaks at 44 and 72 °C, and a shallow one at 98 °C. The dehydration phase could also be observed in the inclusion complex within a narrower temperature range between 25 and 90 °C (Figure 1b).

The first dehydration phase in the uncomplexed α -CD involved a $5.5 \pm 0.5\%$ mass loss (Figure 1c). The dehydration processes occurred through a wider temperature range from 20 to 170 °C as compared to the DSC analysis in which the mentioned processes ended at approximately 120 °C. The total mass loss incurred in these stages of dehydration is equivalent to about 3.2 water molecules that might possibly be bonded interstitially to an α -CD molecule. This amount is slightly lower than the reported 4 interstitially bonded water molecules in α -CD hexahydrate,^{45,47,48} which are readily liberated in the first

phase dehydration, presumably due to the pretreatment of vacuum drying that might have led to a mixture of α -CD hexahydrate and anhydrous α -CD. Meanwhile, the first phase dehydration resulted in a $2.6 \pm 0.3\%$ mass loss in the inclusion complex, which is equivalent to about 1.5 water molecules that might possibly be bonded interstitially to the α -CD (Figure 1d). This may plausibly be partly resulted from the changes in crystalline structure of α -CD triggered by the inclusion complexation with 1-MCP that might have given rise to narrower interstitial space.³⁰ Manor and Saenger also concluded from their diffraction study that the macrocyclic conformation of α -CD ring are different when a water dimer is the guest molecule from that observed with other guest molecules such as 1-propanol, methanol, potassium acetate, and iodine.⁴⁸

A small endothermic reaction that peaked at about 139 °C was recorded in the DSC curve of the uncomplexed α -CD. The similar endothermic peak was related by several groups of researchers to the phase transition of anhydrous α -CD to another form because there was no corresponding mass loss.^{43,46} Nevertheless, a slight corresponding mass loss ($0.2 \pm 0\%$) was found in the TG curve (Figure 1c) of our sample.

Thermal analysis methods can satisfactorily recognize the inclusion complexation of 1-MCP in α -CD. A broad shallow endothermic peak accompanied by a mass loss that took place between 90 and 230 °C was detected in the 1-MCP/ α -CD inclusion complex. This phenomenon was deduced to represent the dissociation of the inclusion complex. The spread of the endothermic dissociation reaction over a great range of temperature may imply the wide difference in the interaction between the 1-MCP molecule and the α -CD cavity in terms of interaction strength. Small mass losses accompanied by small endothermic peaks were recorded at approximately 250–285 °C for both the uncomplexed α -CD and the inclusion complex. The similar phenomenon was reported by Kohata et al.⁴⁹ in their study on the thermal dehydration of α - and γ -CD, according to which, the mass loss was attributed to the loss of very tightly bound water. Berbenni et al.⁴⁷ also confirmed by FT-IR analysis of the evolved gas that the mass loss was due to the release of water molecules. According to Lindner and Saenger, these water molecules were possibly held within the α -CD cavity.⁴⁵ Kohata et al.⁴⁹ and Berbenni et al.⁴⁷ reported respective losses of 0.3 and 0.7 water molecule in their uncomplexed α -CD sample during this dehydration phase, but 0.8 water molecule (a mass loss of $1.3 \pm 0\%$) was liberated from our samples. About 1.6 water molecules (a mass loss of $2.7 \pm 0.2\%$) were lost over this dehydration phase in the inclusion complex. These water molecules could possibly possess as strong interactions with the macromolecules of the inclusion complex as those of the included water molecules with uncomplexed α -CD. However, the orientations of these particular water molecules in the crystalline structure are still unknown.

The typical DSC, TG and derivative thermogravimetry (DTG) curves of 5 mg of 1-MCP/ α -CD inclusion complex recorded at 5 °C/min are shown in Figure 2. The inclusion ratio of the inclusion complex was calculated from the TG data as 0.72 ± 0.01 mol 1-MCP/mol α -CD. The area bounded by the dashed lines represents the temperature range where the dissociation of the inclusion complex occurs, within which the kinetic study on the thermal dissociation of 1-MCP/ α -CD inclusion complex was performed. The kinetic parameters of the dissociation of the inclusion complex were determined calorimetrically as well as gravimetrically based on the DSC and DTG curves, respectively.

Computation of Kinetic Parameters. There are two groups of methods used to analyze nonisothermal solid-state kinetic data—model fitting and model-free methods.⁵⁰ In this study, we employed one of the nonisothermal model-fitting methods—the Freeman and Carroll method that was originally developed assuming a reaction-order model ($f(\alpha) = (1 - \alpha)^n$) as elaborated below.

1-MCP/ α -CD inclusion complex thermally dissociates into its respective solid and gaseous constituents:



Assuming that the above reaction follows an n th-order kinetics and obey the general rate equation⁵¹

$$\frac{d\alpha}{dt} = k(T)(1 - \alpha)^n \quad (2)$$

where $(d\alpha)/(dt)$ (1/s) represents the reaction rate, α the fractional dissociation, k (1/s) the specific dissociation rate constant at temperature T (K), and n the reaction order. As discussed earlier, the patterned peak in Figure 3 represents the dissociation of the inclusion complex. The peak area before an arbitrary temperature, T_i , patterned by the diagonally right up strokes, corresponds proportionally to the amount of dissociated complex at T_i . Meanwhile, the undissociated portion is illustrated by the area after T_i , which is patterned by the diagonally right down strokes. Thus, the dissociated and undissociated fractions of 1-MCP/ α -CD inclusion complex could be described respectively by

$$\alpha = \frac{\Delta H_\alpha}{\Delta H_{\text{Total}}} \quad (3)$$

and

$$1 - \alpha = \frac{\Delta H_{\text{Rest}}}{\Delta H_{\text{Total}}} \quad (4)$$

where ΔH_{Total} is the total peak area obtained by integration of the DSC curve between the commencement and the completion of the dissociation reaction. ΔH_α is the integrated area up to T_i , and ΔH_{Rest} is the area from T_i until the end of dissociation. Substituting (3) and (4) into (2), and rearranging give the below equation, which enables the computation of the specific dissociation rate constant, k at T_i from the DSC curves:

$$k = \frac{1}{\Delta H_{\text{Total}} \left(\frac{\Delta H_{\text{Rest}}}{\Delta H_{\text{Total}}} \right)^n} \cdot \left(\frac{dH}{dt} \right)_i \quad (5)$$

where $(dH/dt)_i$ is the height of the DSC curve at T_i .

Similar procedures were applied for determination of k from the TG data for reciprocal validation. The DTG curves were used for analyses instead of the DSC curves and the specific dissociation rate constant, k could be described by

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$$k = \frac{1}{\Delta m_{\text{Total}} \left(\frac{\Delta m_{\text{Rest}}}{\Delta m_{\text{Total}}} \right)^n} \cdot \left(\frac{dm}{dt} \right)_i \quad (6)$$

where Δm_{Total} is the total peak area obtained by integration of the DTG curve between the commencement and the completion of the mass loss of sample which corresponds to the release of 1-MCP gas, Δm_{Rest} the area from T_i until the end of dissociation, $(dm/dt)_i$ the height of the DTG curve at T_i .

The specific dissociation rate constant, k is a function of temperature, T and can be expressed by the Arrhenius relation as

$$k = k_0 \cdot \exp\left(-\frac{E_D}{RT}\right) \quad (7)$$

where k_0 is the pre-exponential factor, E_D the apparent activation energy of dissociation, R the universal gas constant and T the absolute temperature.

The k values were determined between 30% ($\alpha = 0.3$) and 70% ($\alpha = 0.7$) of the total peak area. An Arrhenius plot of the logarithmized k ($\ln k$) versus the inversed temperature ($1/T$) was employed as a tool for determination of the reaction order, n . The n value was varied in the range 0.5–3 by an interval of 0.5, and the one that gave the most linear plot was determined as the reaction order. The apparent activation energy of thermal dissociation, E_D for the 1-MCP/ α -CD inclusion complex analyzed at 5 °C/min and the coefficient of determination, R^2 of the linear regression of Arrhenius plot were tabulated in Table 1. The data represent the mean values of three independent runs calculated for different n values. Except for the case when $n = 0.5$, no alteration of R^2 was observed down to the second digit after the decimal point with all R^2 values greater than 0.99, indicating equally satisfactory linear regression of the Arrhenius plots. Because no reactant other than 1-MCP/ α -CD inclusion complex is present in the nonisothermal dissociation reaction, it could reasonably be regarded as a unimolecular reaction which depends only on the concentration of the inclusion complex. Thus, the mechanism of the solid-state dissociation reaction would plausibly be best described by the unimolecular decay law ($f(\alpha) = (1 - \alpha)$) with $n = 1$. Generally, rather close values of E_D were obtained by both the TG and the DSC methods. At $n = 1$, the E_D were calculated to be 42.3 ± 0.6 and 39.8 ± 1.0 kJ/mol from the respective methods. These close values prove that both calculation methods for the determination of E_D are reliable. As shown in Figure 4, although the k values calculated from the TG and the DSC methods differ from one another, the inclination of the Arrhenius plots was almost similar thus yielding close E_D values.

Heat Treatment of Inclusion Complex. The inclusion complex was subjected to heat treatment to vary its inclusion ratio. After being heated at 120 °C for 30, 90 and 240 min, the inclusion ratio of the inclusion complex was reduced to values as tabulated in Table 2. For each treatment time, the data represent the mean value of nine independent measurements, which consist of triplicate measurements at three heating rates of 5, 7, and 10 °C/min. The inclusion ratio decreased with heat treatment time in a quadratic manner. Meanwhile, a surprising phenomenon was observed where the moisture content of the inclusion complex increased with heat treatment time, in inverse proportion to the inclusion ratio. The well linear correlation between the moisture content and inclusion ratio with a coefficient of determination of 1.00, as demonstrated in Figure

TABLE 1: Variation of Apparent Activation Energy of Dissociation with Dissociation Reaction Order, n , and Coefficient of Determination, R^2 of Linear Regression of the Arrhenius Plot^a

n	TG		DSC	
	E_D [kJ/mol]	R^2 [–]	E_D [kJ/mol]	R^2 [–]
0.5	18.5 ± 0.7	0.95 ± 0.01	15.1 ± 0.9	0.91 ± 0.02
1.0	42.3 ± 0.6	1.00 ± 0	39.8 ± 1.0	1.00 ± 0
1.5	66.1 ± 0.7	1.00 ± 0	64.5 ± 1.2	1.00 ± 0
2.0	89.9 ± 0.8	1.00 ± 0	89.2 ± 1.3	1.00 ± 0
2.5	113.7 ± 0.9	1.00 ± 0	133.9 ± 1.5	1.00 ± 0
3.0	137.5 ± 1.1	1.00 ± 0	138.6 ± 1.7	1.00 ± 0

^a Sample size is 5 mg, heating rate is 5 °C/min.

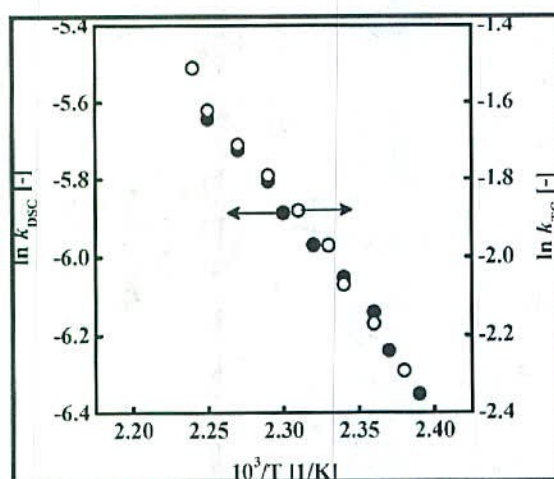


Figure 4. Arrhenius plots of the specific dissociation rate constant, k calculated using (5) and (6) from DSC and TG data, respectively. DSC and TG were performed on 5 mg samples at 5 °C/min.

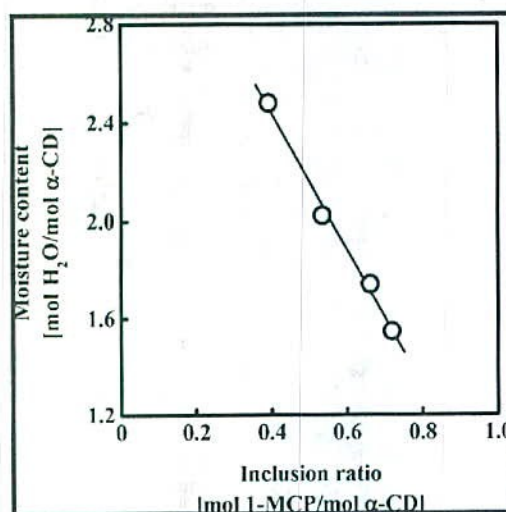


Figure 5. Moisture content as a function of inclusion ratio of inclusion complexes after heat treatment at 120 °C.

5, plausibly indicated the displacement of a released 1-MCP molecule from the α -CD cavity by approximately 2.8 water molecules. This result is in rather good agreement with the reported 2.5 water molecules possibly residing in the cavity of uncomplexed α -CD.^{45,47}

Effect of Inclusion Ratio on Dissociation. Five milligrams of the heat-treated inclusion complexes with inclusion ratios ranging from 0.39 to 0.66 mol 1-MCP/mol α -CD and the untreated inclusion complex were subjected to TG and DSC analyses at heating rates of 5, 7, and 10 °C/min. The apparent activation energy of dissociation, E_D obtained from the TG and

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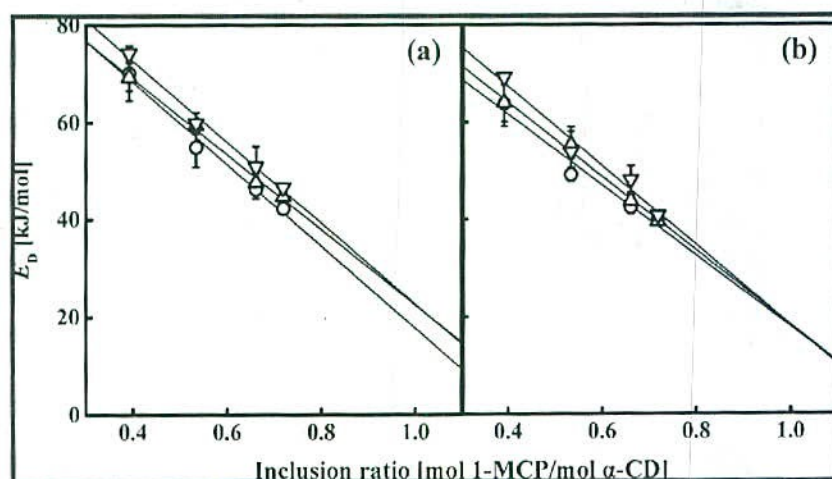


Figure 6. Apparent activation energy of dissociation calculated from TG (a) and DSC (b) data for inclusion complexes of various inclusion ratios. The solid lines are the linear regression and extrapolation to the inclusion ratio of 1 mol 1-MCP/mol α -CD for estimation of the "true" E_D . Heating rate: $\circ$, 5 $^{\circ}\text{C}/\text{min}$; Δ , 7 $^{\circ}\text{C}/\text{min}$; ∇ , 10 $^{\circ}\text{C}/\text{min}$.

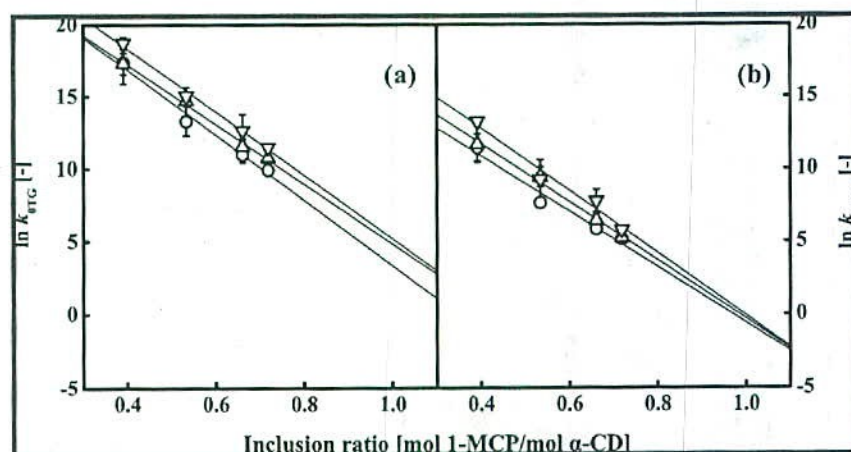


Figure 7. Logarithmized pre-exponential factor calculated from TG (a) and DSC (b) data for inclusion complexes of various inclusion ratios. The solid lines are the linear regression and extrapolation to the inclusion ratio of 1 mol 1-MCP/mol α -CD for estimation of the "true" $\ln k_{\text{OTG}}$ and the "true" $\ln k_{\text{ODSC}}$. Heating rate: $\circ$, 5 $^{\circ}\text{C}/\text{min}$; Δ , 7 $^{\circ}\text{C}/\text{min}$; ∇ , 10 $^{\circ}\text{C}/\text{min}$.

TABLE 2: Inclusion Ratio and Moisture Content of 1-MCP/ α -CD Inclusion Complex after Heat Treatment at 120 $^{\circ}\text{C}$

treatment time [min]	inclusion ratio [mol 1-MCP/mol α -CD]	moisture content [mol $\text{H}_2\text{O}/\text{mol } \alpha\text{-CD}$]
0	0.72 ± 0.01	1.55 ± 0.15
30	0.66 ± 0.01	1.74 ± 0.27
90	0.53 ± 0.01	2.02 ± 0.27
240	0.39 ± 0.02	2.48 ± 0.34

the DSC data collected at the prescribed heating rates was plotted as a function of the inclusion ratio of the inclusion complexes as illustrated in Figure 6a,b, respectively. Both the TG and the DSC data revealed a generally similar trend, where E_D heightened as the inclusion ratio diminished, implying higher complex stability and as well a growing dependency of the specific dissociation rate constant, k on temperature at lower inclusion ratios. The possible explanation for the phenomenon would be that the heat treatment might have forcefully expelled 1-MCP molecules from the α -CD cavities in a selective manner dependent on the strength of the interaction and thus the remaining 1-MCP molecules needed progressively higher energy for dissociation. The $\ln k_{\text{OTG}}$ and $\ln k_{\text{ODSC}}$, when plotted against the inclusion ratio, revealed a trend comparable to that of E_D (Figure 7).

The calculated values of E_D , $\ln k_{\text{OTG}}$, and $\ln k_{\text{ODSC}}$ became less clustered around the mean values with decreasing inclusion

ratio, which are shown by the diverging patterns of the data, especially from DSC analysis (Figures 6b and 7b). The increasingly scattering results at lower inclusion ratios indicated the increasing susceptibility of dissociation to the effect of heating rate with decreasing inclusion ratio, which might possibly be related to the progressive increase of energetically diversified complex molecule species during the heat treatment process, to which the inclusion complex was subjected for varying its inclusion ratio.

By noting that the E_D at 0.72 mol 1-MCP/mol α -CD were least scattered around the respective mean values of 44.6 ± 2.0 and 40.0 ± 0.9 kJ/mol for TG and DSC, it is reasonable to deduce that the E_D will continue to converge until the inclusion ratio reaches 1 mol 1-MCP/mol α -CD, at which the unimolecular decay law is anticipated to best describe the dissociation reaction. The inclusion complex with an inclusion ratio of 1 mol 1-MCP/mol α -CD is an ideal inclusion complex, in which every α -CD cavity is occupied by a molecule of 1-MCP. We speculate that this imaginary inclusion complex could possibly be almost energetically uniform, which would account for the converging patterns of E_D toward 1 mol 1-MCP/mol α -CD. Thus, the estimated E_D by regression and extrapolation to the inclusion ratio of 1 mol 1-MCP/mol α -CD would plausibly represent the "true" E_D . On the basis of the assumption, the "true" E_D was calculated by averaging the E_D values determined

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by regression analysis and extrapolation to 1 mol 1-MCP/mol α -CD for different heating rates (solid lines in Figure 6). Both the TG and DSC methods revealed rather close values of "true" E_D of 20.9 ± 2.8 and 18.1 ± 0.2 kJ/mol, respectively. The "true" $\ln k_{0TG}$ and the "true" $\ln k_{0DSC}$ were also determined by the same method (solid lines in Figure 7), unveiling their respective values of $+4.5 \pm 1.0$ and -0.3 ± 0.3 .

It is commonly known that despite the comparatively higher resolution of reaction phases by low heating rates, the obtained results tend to deviate largely, most probably, for instance in the case of heat stimulated dissociation, due to the readsorption of the dissociated gas. Nevertheless, high heating rates also pose a similar problem of scattering results owing to higher possibility for the establishment of a high temperature gradient in the sample. However, in our case, the latter is not likely as the thermal lag for the heating rate of 10 °C/min was recorded at a mere 1.2 °C.

Conclusions

The thermal dissociation kinetics of 1-MCP/ α -CD inclusion complex was analyzed by a nonisothermal model-fitting method—the Freeman and Carroll method. The dissociation of every 1-MCP molecule by heat treatment at 120 °C was accompanied by an adsorption of 2.8 water molecules. This may imply the important role played by water molecules in the dissociation of 1-MCP/ α -CD inclusion complex. Because temperature and humidity are the two main factors that affect the stability of an inclusion complex during storage, the dissociation of 1-MCP/ α -CD inclusion complex in response to storage humidity is also of interest. The research study on dissociation with respect to storage humidity is currently underway. Both TG and DSC were fairly reliable for the determination of E_D although the k values were significantly different between the two methods. The temperature dependency of the thermal dissociation reaction of 1-MCP/ α -CD inclusion complex exhibits highly reproducible consistency, regardless of the thermal analysis method employed. The "true" E_D was determined to be 20.9 ± 2.8 and 18.1 ± 0.2 kJ/mol for TG and DSC, respectively. The stability of the 1-MCP/ α -CD inclusion complex against dissociation is dependent to a great extent upon the initial inclusion ratio of the inclusion complex. The information on the heat stability of the 1-MCP/ α -CD inclusion complex is expected to be useful for the viability assessment of unit processes that involve heat treatment during the development of new ways of application for the inclusion complex.

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Molecular Characterization of Wax Isolated from a Variety of Crude Oils

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Petroleum waxes from sixteen different crude oils were analyzed after isolation from the crude in a two-step process. These waxes were characterized molecularly to aid in the investigation of the effect of wax type and quantity on water-in-crude oil emulsion stability. The techniques used to gather information about the waxes included elemental analysis, FTIR spectroscopy, field desorption mass spectroscopy, ^1H NMR spectroscopy, ^{13}C NMR spectroscopy, gel permeation chromatography, and differential scanning calorimetry. Two distinctly different types of petroleum waxes were discerned, microcrystalline and paraffinic. Microcrystalline waxes are aliphatic hydrocarbon compounds containing a substantial amount of branches and rings. They have large molecular weight ranges from 300 to 2500 amu and are gel-like in appearance. The rings give rise to a decreased H/C ratio relative to paraffinic waxes, often as low as 1.85. Because of the large number of different compounds and the lack of large-scale crystallinity, a distinct melting regime is not observed. FTIR spectroscopy determined that these waxes contained approximately 55% straight chain methylene, that is, methylene groups directly attached to each other. Five waxes exhibited these characteristics and thus were considered typical microcrystalline waxes. Paraffin waxes are hydrocarbon chains with few or no branches and H/C ratios between 1.96 and 2.05. They have distinct melting regions and narrow molecular weight ranges between 350 and 600 amu. They contained 63–78% straight chain methylene as gauged by FTIR spectroscopy. Six of the remaining eleven waxes exhibited all the characteristics of this paraffin category while the other five were mixtures of microcrystalline and paraffinic wax.

Introduction

Wax has been important in a variety of industries for a number of years. It has been used for everything from candles, polishes, and crayons to container coatings and impregnating paper.^{1,2} Much of this wax comes from petroleum. While wax is useful after separation from the crude, before separation it can cause or magnify a number of problems in the processing of petroleum. It can aid emulsification of the crude in the production and refining of crudes, as well as in oil spills.^{3,4} Since the wax precipitates out at low temperatures, it can cause plugging in drilling equipment and pipelines undersea and in the arctic.⁵ Because of its high viscosity, it can cause pumping problems in pipelines and when emptying tankers in regions where the water temperature is low. Our research team has focused on the role of waxes in the formation of emulsions in production and refining. These emulsions are difficult to study because of the variety of different crudes and crude waxes, the complexity of crude oil, and the lack of knowledge of the precise mechanism whereby wax stabilizes emulsions.

Numerous papers on water-in-crude-oil emulsion stability have been published recently, but the majority have focused on the role of resins and asphaltenes in the stabilization of water-in-oil emulsions.^{6–10} Research has indicated that these emulsions are stabilized by a rigid film that surrounds the water droplet.^{11–13} This film is comprised of various materials, primarily resins and asphaltenes. These are general terms for two different solubility classes of polar surface active molecules found adsorbed at the interface. These materials are the primary emulsion stabilizers for water-in-oil emulsions. Various solids have also been scrutinized for their capability to act as secondary stabilizers in these emulsions. One of the most common solids implicated is wax.^{3,4,11,14–17} These waxes are typically submicrometer particulate solids that can adsorb resins

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Table 1. Characteristics of the Whole Crudes

crude, full name	crude, abbrev	% organic precipitate	% wax	% resins	% asphaltenes
Malu Isan	MI	8.48	5.80	4.87	0.18
South China Sea	SCS	42.95	32.45	6.05	3.47
B6 CUSA	B6	18.76	1.08	12.05	13.13
Arab Berri	AB	3.80	1.68	3.49	0.68
Alaska North Slope	ANS	7.30	1.54	9.47	3.35
Statfjord	SF	11.10	8.10	4.02	0.09
B4 CUSA	B4	18.23	1.53	12.22	13.55
Maya	Maya	25.09	3.85	10.96	11.54
Arab Heavy	AH	12.27	1.54	9.56	8.27
Canadon Seco	CS	31.80	16.10	8.94	7.51
Gulf of Mexico	GM	12.5	7.76	5.02	0.32
Alba	Alba	10.10	1.54	10.12	1.64
Bay Marchand	BM	5.57	1.20	6.96	0.16
THUMS-1	T1	11.00	1.35	18.69	5.09
THUMS-2	T2	8.10	1.00	12.48	3.31
San Joaquin Valley	SJV	10.46	0.69	20.26	4.57

and asphaltenes to become interfacially active or are themselves interfacially active. They are then capable of adsorbing at the water-oil interface, thus stabilizing the water droplet. They play a supporting role in emulsion stability, but their importance is extremely dependent on temperature. Cooling rate and emulsion formation temperature can determine whether wax will play a role in an emulsion's stability or not.^{4,16}

Although wax has been mentioned as a possible emulsion stabilizer for years, its role in emulsion stability has not been extensively studied. Current wax research is primarily aimed at understanding the phenomenon of wax precipitation at low temperatures.¹⁷⁻²¹ However, wax has recently assumed more importance in crude oil emulsion stability studies. Still, data are normally limited to wax precipitation temperature and wax content of the crude in question.¹⁸ Even from these limited data, some conclusions can be drawn about the role of wax in emulsion stability. One group of researchers found a correlation between the amount of wax present in the crude and the emulsion stability which had wax content increasing as emulsion stability increased.¹⁷ However, they did not examine any properties of the wax other than the total wax content of the crude. Others have added or removed wax to examine the effect on emulsion stability.^{3,4,14,16,22} However, the wax used by these researchers has never been characterized prior to its use in these studies.

Wax has been characterized previously by some researchers. Branthaver and Holmes characterized wax from tar sand bitumens.^{23,24} Branthaver found H/C ratios of 1.9, sulfur contents of 0.07 wt %, and nitrogen contents of 0.18–0.38 wt % for waxlike substances. The melting point was between 91 and 96 °C. Solid-state ¹³C NMR showed only methyl and methylene groups, but methine could not be differentiated from methylene peaks. FTIR indicated a large peak at 720 cm⁻¹ which was indicative of long straight methylene chains. The mass spectrum indicated a mixture of *n*-alkanes with carbon numbers extending past 60. Holmes characterized wax separated from crude oil in a variety of ways. The wax FTIR spectrum showed a strong absorbance at 720 cm⁻¹. The ¹³C NMR spectrum displayed a prominent peak at 29.7 ppm, again a strong indication of long straight chain methylene. Elemental analysis indicated an H/C ratio of 1.86. Field desorption mass spectroscopy indicated an average molecular weight of 744 Da. Handoo analyzed petroleum wax from an

unnamed source.²⁵ The H/C ratio was between 1.77 and 2.21 with molecular weights between 355 and 915 Da. The melting points were between 53 and 86 °C.

The purpose of this study was to characterize the wax obtained from sixteen different crudes. They have resin contents between 3.5 and 20%, asphaltene contents between 0.09 and 13.5%, and wax contents between 0.7 and 32% as shown in Table 1. The waxes were precipitated via a modified Burger's method.²⁶ The precipitated material was then fractionated to separate the wax from the polar material that had coprecipitated. The resulting wax was analyzed by elemental analysis to determine the H/C ratio and heteroatom distribution (%S, %N, %O). The waxes were also analyzed via Fourier transform infrared (FTIR) spectroscopy to determine long straight chain methylene content. Field desorption mass spectroscopy was performed to determine the mass range of the waxes. Differential scanning calorimetry (DSC) was performed to determine the melting temperatures and enthalpies of the waxes. Information on the structure was derived from proton and carbon NMR. Gel permeation chromatography was performed to supplement the mass spectra data and to allow a better examination of the mass distribution. From these data, percentage methyl, methylene, and methine carbon were calculated and used to create average molecular structures.

Materials and Methods

Materials. Sixteen crude oils from various fields around the world were investigated. Chevron supplied B4 CUSA (B4)

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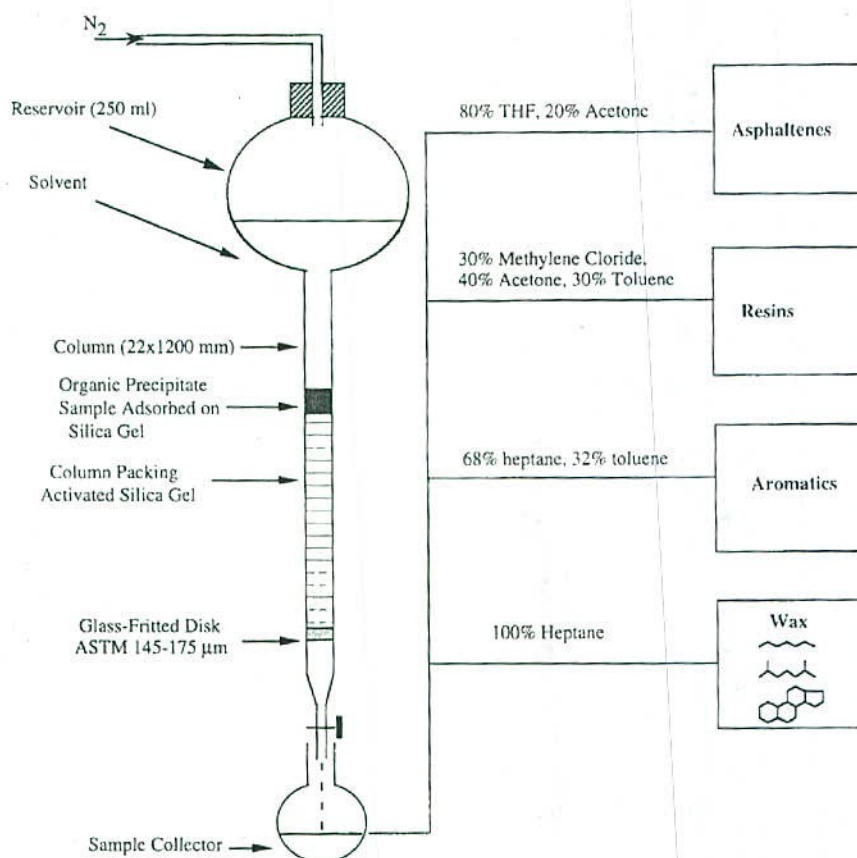


Figure 1. Sequential elution chromatography setup for separating wax precipitate into waxes, aromatics, resins, and asphaltenes.

and B6 CUSA (B6) from California, Alba (Alba) from the North Sea, Bay Marchand (BM) from off the Louisiana Gulf Coast, and Malu Isan (MI) from Nigeria. Texaco contributed Arab Berri or Arab Extra Light (AB) from Saudi Arabia, South China Sea (SCS) from China, and Gulf of Mexico (GM) from the U.S. Gulf of Mexico. Mobil furnished Arab Heavy or Safaniya (AH) from Saudi Arabia and Statfjord (SF) from the North Sea. Shell provided San Joaquin Valley (SVJ) from California, Maya (Maya) from Mexico, and Canadon Seco (CS) from Venezuela. BP supplied Alaska North Slope (ANS) from Alaska. THUMS-1 (T1) and THUMS-2 (T2) from Santa Monica Bay were provided by Arco.

The methylene chloride, acetone, toluene, tetrahydrofuran (THF), pyridine, and *n*-heptane used were HPLC grade. The petroleum ether was Optima grade. The silica gel was Fisher Scientific chromatographic grade, 35–60 mesh, Davisil. The filter paper used was a Whatman 934 AH glass microfiber filter. All were purchased from Fisher Scientific. The sequential elution chromatography apparatus was made by University Research Glassware. All other glassware were purchased from Fisher Scientific.

Solids Precipitation. The initial stage of wax separation used a method first proposed by Burger in 1981 and subsequently modified by other researchers.^{14,18} For each precipitation, 5 g of crude oil was dissolved in 35 mL of petroleum ether. When the oil was dissolved, 110 mL of acetone was added. The sample, all of the filtration apparatus, and a wash solvent composed of acetone and petroleum ether (3:1 v/v) were cooled for 2 h at -20°C . The sample was then filtered (Whatman 934 AH glass microfiber filter) through a Buchner funnel ($d = 15\text{ cm}$) into a 1000 mL vacuum flask on which a slight vacuum was drawn. The retentate was washed with the wash solvent until clear solvent flowed. It was then rinsed into a flask with methylene chloride, and the solvent evaporated. The retentate was transferred to a tared vial and dried in a nitrogen-flushed vacuum oven at 60°C for 48 h.

Sequential Elution Chromatography. Elemental analysis established that the precipitate contained more than saturated hydrocarbons, mainly through the discovery of a low H/C ratio and significant heteroatom content. A second separation—sequential elution chromatography—was therefore performed. The material recovered in the first fraction of this chromatographic separation was the wax. One gram of organic precipitate was transferred to a flask. The material was adsorbed onto 30 g of silica gel (activated at 120°C in a nitrogen-flushed oven) using methylene chloride. It was stirred for 24 h, before solvent removal by rotary evaporation. The adsorbed sample was next dried in a nitrogen-flushed oven at 60°C for 48 h. It was charged to a standard chromatography column. These Pyrex columns were constructed with a 250 mL reservoir attached to a 14/20 joint, a 22 mm o.d. column approximately 1 m long, a fritted disk (ASTM 145–175 μm), and a detachable Teflon stopcock with removable Pyrex tip. A diagram of a packed sequential elution chromatography column is supplied in Figure 1. The material that eluted in heptane prior to the heptane's color change was considered the wax. The aromatics were eluted in 400 mL of heptane and toluene (17/8 v/v). Then the resins were eluted in 350 mL of a mix of toluene, acetone, and methylene chloride (3/4/3 v/v). Finally a fraction called asphaltenes were eluted in 350 mL of THF and acetone (4/1 v/v). The silica was then placed in pyridine for several days and the desorbed material added to the asphaltenes. The solvent was removed from all the fractions except the asphaltenes via rotary evaporation, and they were dried in a nitrogen-flushed vacuum oven at 70°C for 48 h. Because of THF oxidation, the solvent was removed from the asphaltenes via sparging with argon to evaporate the solvent before drying.

Elemental Analysis. Elemental analyses (C, H, N, S, and O) of these precipitates and their fractions were obtained with the use of a Perkin-Elmer 2400 Series II CHNS/O analyzer. A combustion method was used to determine C, H, N, and S

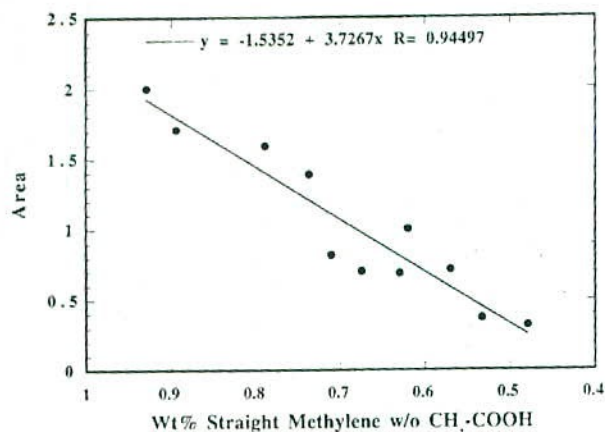


Figure 2. FTIR calibration plot for 720 cm^{-1} peak (10 g/L in cyclohexane solution) using eleven alkanes, acids, and alcohols: octane, octanoic acid, decanoic acid, decane, decanol, undecylenic acid, lauric acid, myristic acid, palmitic acid, eicosane, and triacontane.

content while the sample was pyrolyzed in a helium/hydrogen mixture to determine O content. In both methods, the homogenized gases were depressurized through a column where they were separated in a stepwise manner and detected as a function of their thermal conductivities. A known standard was first analyzed to calibrate the analyzer. The calibration factor calculated from this analysis was then used to determine unknowns. The system was specified for a precision of ± 0.3 wt % for each element. With multiple samples, the reproducibility was found to decrease to ± 0.15 wt %. The samples were weighed with a Perkin-Elmer AD-6 Ultramicrobalance.

FTIR Spectroscopy. In the Fourier transform infrared (FTIR) studies, an RFX-65 FTIR spectrometer equipped with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector was used to obtain the spectra from dilute solution (10 g/L) of cyclohexane through a variable path length KCl cell with a 0.2 mm spacer. The background spectrum of pure cyclohexane was integrated and automatically subtracted from the sample spectra. Sixty-four scans were taken of each sample recorded from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode. Peak position and area were determined by using available software packages. The area under the absorption band of interest was used to evaluate quantitative estimates of functional group concentrations via Beer's law. The Beer's law constant was calculated from an average of the constants for a variety of straight chain hydrocarbons from decane to triacontane to palmitic acid. The calibration curve is shown in Figure 2. Spectra were recorded using the linear transmission mode of the spectrometer. Absorption band areas having the units " cm^{-1} " were then calculated by a software package integration feature.

Mass Spectroscopy. The molecular weights were found using field desorption mass spectroscopy. The sample was coated onto a silicon emitter. The cathode potential was between 1.5 and 2 kV, and the emitter currents varied from 0 to 30 mA at 2 mA/min. The detector gain was 1.0–1.4 kV, and the control field was magnetic. The acceleration voltage was 10.0 kV, and the mass scan range was 0–3000 Da. The mass spectrometer was a JEOL HX-110 double focusing mass spectrometer, Nier-Johnson configuration. The mass spectrometer was controlled by a Hewlett-Packard 9000 series 400 Unix workstation running HP/UX 8.0.

Gel Permeation Chromatography. Molecular weight distributions were found using gel permeation chromatography. Three columns, 100, 10, and 1 kDa, were linked in series. The stationary phase was a Gordi gel cross-linked polymer styrene divinyl benzene mixed bead. The mobile phase was

Table 2. Comparison of Resins and Asphaltenes Separated via Wax Precipitation and SEC versus Asphaltene Precipitation and SEC

crude	% resins		% asphaltenes		R/A ratio		asp. H/C ratio	
	wax	asp.	wax	asp.	wax	asp.	wax	asp.
MI	0.82	4.87	0.77	0.18	1.06	27.06	1.47	1.42
B6	9.14	12.05	6.49	13.13	1.41	0.92	1.23	1.23
AB	0.74	3.49	0.67	0.68	1.11	5.13	1.15	1.02
ANS	2.33	9.47	2.52	3.35	0.92	2.83	1.12	1.08
B4	7.48	12.22	7.35	13.55	1.02	0.90	1.20	1.23
AH	2.47	9.56	4.39	8.27	0.56	1.16	1.15	1.11
Alba	3.16	10.12	2.94	1.64	1.07	6.17	1.23	1.15
BM	1.82	6.96	1.46	0.16	1.25	43.50	1.22	1.26
SJV	4.25	20.26	3.82	4.57	1.11	4.43	1.20	1.17

THF. Sample concentrations were 3–6 mg/mL, and 100 μL was injected at a time. The flow rate was 1.5 mL/min, and the column temperature was 40 $^{\circ}\text{C}$. The data were collected by an ERC Inc. RI detector with a range setting of 8. The data were recorded on a strip chart. It was then scanned into the computer and 80–120 representative points were picked via Digimatic, a program that supplies coordinates for points picked off a graph. These data were then plotted to allow the conversion of distance to molecular weight.

Differential Scanning Calorimetry. The melting temperatures and enthalpies were recorded using a Perkin-Elmer DSC 7 differential scanning calorimeter with a PC series thermal analysis system. Calibration was performed using high-purity zinc and indium. The apparatus was continually flushed with nitrogen. The samples weighed between 3 and 20 mg. Each sample was cooled to -25 $^{\circ}\text{C}$ for 10 min. It was then heated at a rate of 10 $^{\circ}\text{C}$ per minute to 175 $^{\circ}\text{C}$. It was rapidly cooled back to -25 $^{\circ}\text{C}$ where it remained for 10 min before reheating to 175 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}$ per minute.

^1H NMR. A General Electric GN 300 Omega NMR spectrometer with a C/H dual 5 mm probe was used for all experiments at an observed frequency of 300 MHz. All samples were approximately 5–10 mg/mL in deuteriochloroform in 5 mm sample tubes. A total of 32 scans was acquired for each sample using an 8 μs ($\sim 90^{\circ}$) pulse and a 4 s delay time between scans.

^{13}C NMR. A General Electric GN 300 Omega NMR spectrometer with a C/H dual 5 mm probe was used for all experiments at an observed frequency of 75 MHz. All samples were approximately 40–80 mg/mL in deuteriochloroform in 5 mm sample tubes. A total of 2560 scans was acquired for each sample using a 10 μs ($\sim 70^{\circ}$) pulse and a 15 s delay time between scans. During the delay, the proton decoupling power was turned off to facilitate more complete relaxation of slowly relaxing carbons.

Results and Discussion

Precipitation. A mixture of acetone and petroleum ether was used to precipitate material from the crude oil at -20 $^{\circ}\text{C}$. The quantity of organic material precipitated varied from 3 to 43% of the crude and was a function of the wax, resin, and asphaltene content of the crude,²⁷ listed in Table 1. While this type of precipitate is sometimes termed wax,²⁶ the relatively low H/C ratio and high heteroatom contents (see Table 2) indicated that polar material had coprecipitated with the wax. Asphaltenes are the most likely coprecipitate, as they are believed to adsorb on wax particles during cooling, interfering with large-scale crystallization.^{18,28} As such, they are pour point depressants and remain

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(28) Ackroyd, G. C.; Cawley, C. M. *J. Inst. Pet.* 1953, 39, 82–111.

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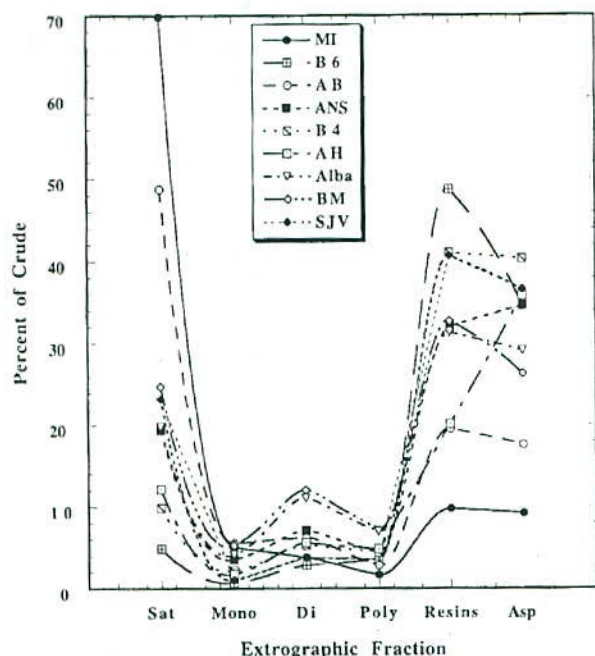


Figure 3. Percent recovery of sequential elution chromatography fractions for organic solids from nine crudes.

in the crystal matrix solubilizing it until the temperature becomes so low that they cannot prevent precipitation.

Sequential Elution Chromatography. Wax was purified following precipitation by sequential elution chromatography with the wax isolated as the first eluted fraction in *n*-heptane.^{27,29} Sequential elution chromatography (SEC) demonstrated that the precipitate was composed mainly of resins, asphaltenes, and wax as shown in Figure 3. Later chromatographies were abbreviated to only elute the wax. As shown in Table 1, the amount of wax isolated did not depend on the amount of material precipitated. However, the crudes with the least asphaltenes did have the highest percentage of wax in the precipitate. No other correlations among the percent resins, asphaltenes, wax, and precipitate have been identified.

McLean²⁷ precipitated asphaltenes in *n*-heptane and then performed sequential elution chromatography upon the filtrate. A comparison of the resins and asphaltenes separated via the two methods is shown in Table 2. The majority of the asphaltenes were recovered in the wax precipitation SEC, but these asphaltenes had generally higher H/C ratios, indicating irreversible adsorption of the more aromatic compounds. Where more asphaltenes were separated from the wax than were found in the whole crude, the additional asphaltenes were likely resins that had strongly adsorbed to the column. The majority of resins did not precipitate with the wax, causing the resin-to-asphaltene ratio (R/A ratio) to be much lower for the organic precipitate than for the whole crude, except for those with extremely high asphaltene contents and low R/A ratios.

The crudes examined here had a variety of wax contents with most having a relatively low percent wax. Other researchers have found wax contents for crude

Table 3. Elemental Analysis Data for the Organic Precipitate and Wax

crude	organic precipitate			wax (saturates)				
	wt % N	wt % S	H/C	wt % N	wt % S	wt % C	wt % H	wt % O
MI	0.24	0.51	1.85	0.00	0.26	85.25	14.59	2.05
SCS				0.00	0.05	86.12	14.23	1.98
B6	1.53	5.57	1.34	0.00	0.50	85.32	13.92	1.96
AB	0.21	2.36	1.64	0.02	0.41	85.96	14.18	1.98
ANS	0.78	2.04	1.39	0.01	0.26	85.64	14.17	1.99
SF				0.00	0.00	86.18	14.06	1.96
B4	1.35	5.38	1.28	0.00	0.48	85.25	13.77	1.94
Maya				0.01	0.30	86.10	13.84	1.93
AH	0.52	5.14	1.39	0.00	0.71	85.53	13.81	1.94
CS				0.00	0.00	86.10	13.82	1.93
GM				0.00	0.30	86.15	13.99	1.95
Alba	0.41	1.86	1.50	0.00	0.37	85.64	13.55	1.90
BM	0.53	1.06	1.41	0.01	0.18	85.93	13.22	1.85
T1				0.00	0.13	86.12	13.41	1.87
T2				0.00	0.16	86.34	13.28	1.85
SJV	1.92	1.36	1.32	0.00	0.14	85.86	13.22	1.85

oils ranging from 0.0 to 16% wax.^{17,18,30} Some North Sea crudes, such as Alba and Statfjord, had wax contents ranging from 2.4 to 14.5% wax.¹⁷ Statfjord agreed with these data, but Alba's wax content was lower.

The amount and physical characteristics of wax determined by other researchers could be a function of the separation method. Both Johansen¹⁷ and Ronningsen¹⁸ precipitated material with Burger's method and then eluted with hexane through a short silica column. However, Johansen first precipitated asphaltenes with *n*-pentane. Agrawal³⁰ precipitated with methyl isobutyl ketone at low temperatures and then decolorized the precipitate by percolation in the molten state through an activated bauxite column. Much of the older research on wax was in fact done on petroleum waxes that were commercially available.

Elemental Analysis. The elemental compositions (carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and oxygen (O)) of the precipitates and the wax obtained from the chromatographic separation were analyzed and collected in Table 3. They indicated that most of the nitrogen and sulfur was contained in compounds with low H/C ratios since in the eluted waxes the percent nitrogen and percent sulfur decreased while the H/C ratio increased. Thus the nonwax material in the precipitate had a low H/C ratio and high sulfur and nitrogen contents, consistent with resins or asphaltenes.

From the H/C ratio and visual inspection, preliminary categorization of the waxes could be made. Those waxes with the lowest H/C ratios, T1, T2, SJV, Alba, and BM, were translucent viscous liquids. These were considered the microcrystalline waxes. Another group was comprised of the waxes with the highest H/C ratios: AB, MI, B6, ANS, SCS, and SF. These waxes were opaque, white, or whitish-yellow solids. The yellow tinge may be a result of contamination by early aromatic elution. These were paraffin waxes. The remaining waxes, Maya, GM, CS, AH, and B4, were tackier solids with H/C ratios between those of the two groups and were mixtures of the two types.

All *n*-alkanes and isoalkanes have H/C ratios above 2 because of their terminal methyl groups. For these

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waxes to have H/C ratios below 2, they must contain rings or double bonds. An H/C ratio of 1.85 for a molecular weight near 400 amu would indicate a $C_{28}H_{52}$ formula with a maximum of 3 rings or 3 double bonds. For the same carbon content, an H/C ratio of 1.94 corresponds to $C_{28}H_{54}$ and a maximum of 2 rings, and one of 1.99 corresponds to $C_{28}H_{56}$ and 1 or 2 rings.

The sulfur content was under 0.5% for virtually all the waxes. Sulfur contents ranged from 0.0 to 0.71% and corresponded to a maximum of 0 to 14% of the wax containing sulfur. The sulfur could be thiol, thiophenic, sulfidic, or sulfoxide, though the FTIR did not indicate the presence of sulfide or sulfoxide. Since most of the organic sulfur found in crude oil is thiophenic, the sulfur in the waxes probably was as well.^{31,32} A C_{28} wax with one thiophenic ring would have an H/C ratio of 1.86, with a formula of $C_{28}H_{52}S$. Thus, sulfur thiophenic rings may account for some of the low H/C ratio. However, as sulfur is present in less than 14% of the wax molecules, it could not account for the majority of the low H/C ratio.

Oxygen in alcohols and ethers has no effect on the H/C ratio, so this decrease in H/C ratio was unrelated to oxygen content unless the oxygen was carbonyl, probably ketone or acid. Even if all the oxygen were carbonyl, it would not account for the majority of the lowered H/C ratio. The 1H NMR indicated the presence of ketone but negligible alcohol or ether. Thus the oxygen was mainly ketone or acid.

Some elemental analysis has been done on wax by other researchers, but remarkably little was on wax precipitated from petroleum. Holmes and Branthaver both examined bitumens, a close relative of petroleum. Holmes used desorptive Soxhlet extraction with heptane followed by complexation chromatography with cyclohexane to elute the saturates. The two bitumens examined had saturate fractions with H/C ratios of 1.86 and 2.07. Branthaver used Soxhlet extraction with benzene and ethanol followed by dewaxing via toluene/2-butanone at 0 °C to obtain wax. These waxes had H/C ratios of 1.87 and 1.94, a nitrogen content of 0.38%, and a sulfur content of 0.07%. The sulfur content was within the range found here, while the nitrogen content was higher. The only H/C ratio for a petroleum wax was found by Ackroyd.²⁸ The wax was separated by deasphalting the crude with benzene and adsorbing the remainder onto alumina. The pentane extract that was insoluble in acetone and that did not form a urea complex had an H/C ratio of 1.74. Since urea complexes are formed with *n*-alkanes, this H/C ratio is for branched and cyclic alkanes, thus its low H/C ratio in comparison with those here. The fraction that formed a complex with urea had an H/C ratio of 2.01, much closer to what is expected for a *n*-alkane or isoalkane.

FTIR. FTIR transmission spectra were obtained of all the waxes. The peak at 720 cm^{-1} has long been associated with long straight chain methylene,^{23,24} but no one has reported a Beer's Law constant or determined percent straight chain methylene. Few researchers have examined this peak because it is often obscured

Table 4. Weight Percent Straight Chain Methylene Obtained by FTIR, Melting Peaks, and Heats of Fusion Obtained by DSC for All Waxes

crude	% straight chain methylene	ΔH (cal/g)	peak (°C)
MI	77.91	33.19	51.34
SCS	66.38	26.13	43.43
B6	66.70	25.22	57.98
AB	68.98	17.00	47.94
ANS	62.71	19.54	46.89
SF	66.17	19.03	32.19
B4	65.30	18.00	45.82
Maya	63.40	13.37	39.42
AH	64.21	9.88	42.83
CS	60.93	11.70	30.10
GM	61.80	9.20	32.55
Alba	60.03	3.11	36.63
BM	55.49	0.88	34.06
T1	55.52	10.11	36.96
T2	53.12	3.02	32.90
SJV	51.70	0.25	30.60
eicosane		58.56	39.27

by solvent or by window opaqueness. This peak is associated with straight chain methylene at least four units long. Squalane, a branched hydrocarbon, had no peak at 720 cm^{-1} , but did have a peak at 735 cm^{-1} . This may be where methyl branched methylene chains absorb. In many cases, the percentage transmittance did not return to the baseline immediately after the 720 cm^{-1} peak, probably due to branches as demonstrated by squalane's 735 cm^{-1} peak. Therefore a symmetric peak was created by reflecting the peak through its highest point. This FTIR peak had a very small extinction coefficient of only 94.9 L/mol cm^2 . Consequently, the uncertainty in this measurement is large.

A variety of compounds were used to calculate the Beer's law constant for straight chain methylene. Because of the wax's heterogeneity, acids, an alcohol, and *n*-paraffins were used in the determination of the Beer's law constant. Octane, octanoic acid, decanoic acid, decane, decanol, lauric acid, myristic acid, palmitic acid, undecylenic acid, eicosane, and triacontane were used in this calculation. The best fit for the data was if the methylene group next to the acid group was not included.

From this FTIR measurement, the waxes were gauged to contain between 50 and 80% straight methylene chains at least 4 methylene units long (see Table 4). In addition, the waxes likely contain shorter chains, rings, and branches. On the basis of the measurements, SJV, Alba, BM, T1, and T2 fell at one end of the spectrum with 60% or less straight methylene. AB, B6, MI, SCS, and SF were at the other end with more than 66% straight methylene.

Mass Spectroscopy. The mass spectroscopy data definitively separated the waxes into the two categories mentioned previously. Several of the waxes in this study (AB, NS, B6, MI, SCS, and SF) had mass spectra reflecting a normal distribution of *n*-alkane or isoalkane hydrocarbons. The peaks were evenly spaced, 14 amu apart, covering a range from 350 to 600 as shown in Figure 4. However, five of the waxes, SJV, Alba, BM, T1, and T2, had very different mass spectra. These spectra began at near 350–400 amu but continued to 2000–3000 amu as in Figure 5. On the basis of the principles of the field desorption method, it is presumed that the profile shown accurately reflects the mass

(31) Gorbaty, M. L.; George, G. N.; Kelemen, S. R. *Fuel* **1990**, 69 (8), 945–949.

(32) Waldo, G. S.; Carlson, R. M. K.; Moldovan, J. M.; Peters, K. E.; Penner-Hahn, J. E. *Geochim. Cosmochim. Acta* **1991**, 55 (3), 801–814.

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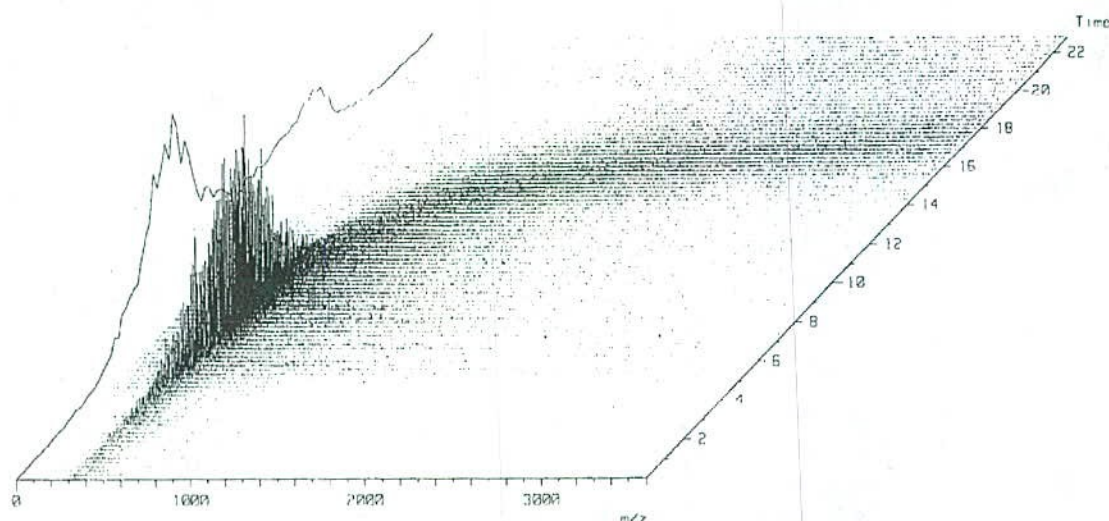


Figure 4. Malu Isan mass spectrum (field desorption mass spectroscopy) showing paraffin character.

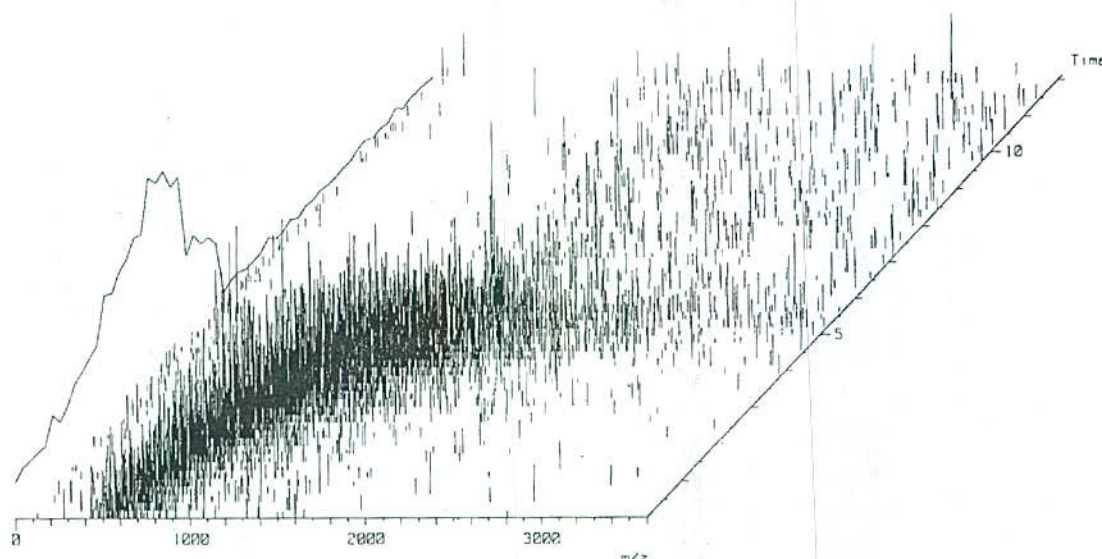


Figure 5. San Joaquin Valley mass spectrum (field desorption mass spectroscopy) showing microcrystalline character.

distribution of parent ions or molecules. Also, these waxes were not a simple 14 amu apart in the lower mass region. Some waxes, B4, Maya, GM, AH, and CS, were largely paraffinic in the lower mass regions, but contained a significant amount of higher molecular weight compounds as shown in Figure 6.

A few researchers have used a variety of methods to obtain molecular weight data for waxes. The data varied considerably, and only an average molecular weight was recorded. Some listed average molecular weights between 250 and 500 amu,^{2,18,33} or between 500 and 800 amu,^{5,23,34} or even higher, between 814 and 1078 amu.²⁸

Gel Permeation Chromatography (GPC). GPC was performed to determine an accurate median molecular weight. The median molecular weight was between 300 and 700 amu for all the waxes. As shown in Figures 7 and 8, most tailed off before 2000 amu. This may be incorrect as the baseline was determined visually, and thus a relatively small amount of material

could have been obscured by baseline variability. A few of these waxes, SCS, SF, MI, and B6, had a relatively narrow molecular weight range. Some also had small peaks near 100–200 amu which may be the result of contamination.

For SJV, Alba, BM, T1, and T2, the plots were inconsistent with the mass spectroscopy. Either the higher molecular weight compounds did not alter the refractive index much or field desorption mass spectroscopy falsely exaggerated the higher molecular weight peak heights. A propagation of error analysis was performed on the data. Even though the error depended on the molecular weight, the deviation was only 200 at 2000 Da. Thus, errors in reading the data cannot account for all the inconsistency.

Differential Scanning Calorimetry. The differential scanning calorimetry data confirmed the separation of the waxes into two distinct groups (see Table 4). SJV, Alba, T1, T2, and BM had very indistinct peaks even at significantly higher sample weights than those necessary for the other waxes as shown in Figure 9. These waxes melt over such a large region that no real discernible melting range could be found, as the enthal-

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(34) Mazee, W. M. *Modern Petroleum Technology*; Applied Science Publishers Ltd.: Barking, 1975; pp 782–802.

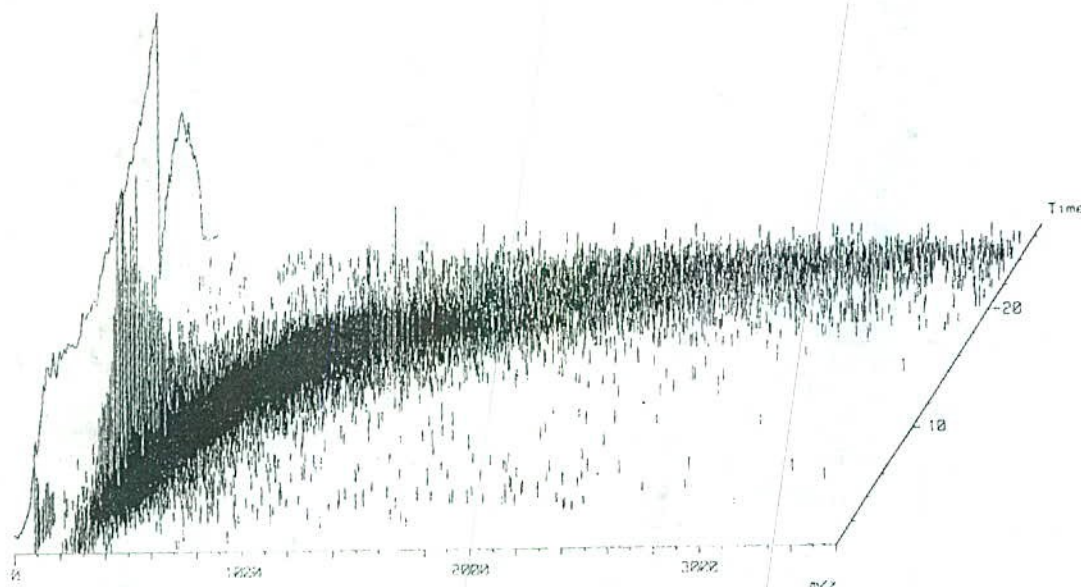


Figure 6. Canadon Seco mass spectrum (field desorption mass spectroscopy) showing a mixed paraffin-microcrystalline character.

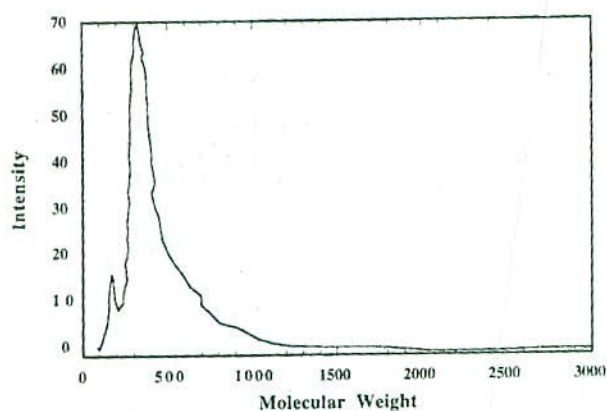


Figure 7. Malu Isan gel permeation chromatography for 0-3000 Da.

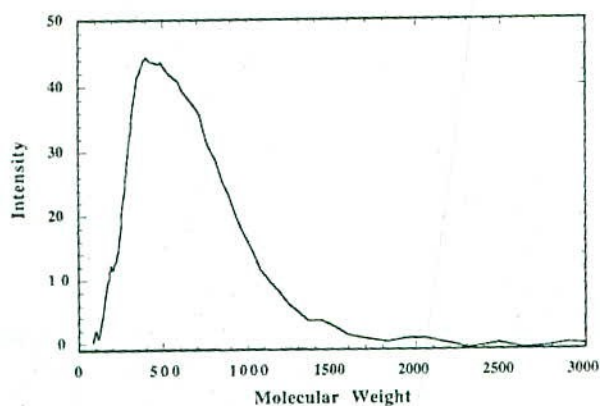


Figure 8. San Joaquin Valley gel permeation chromatography for 0-3000 Da.

pies found for the slight bulges seen are too small to be those of wax. The absence of a true melting region for these waxes has several possible explanations. The heat of fusion decreases with the increased isoparaffin content.³⁵ Thus these waxes could be mainly composed of isoparaffins or cycloparaffins. Also, the higher the

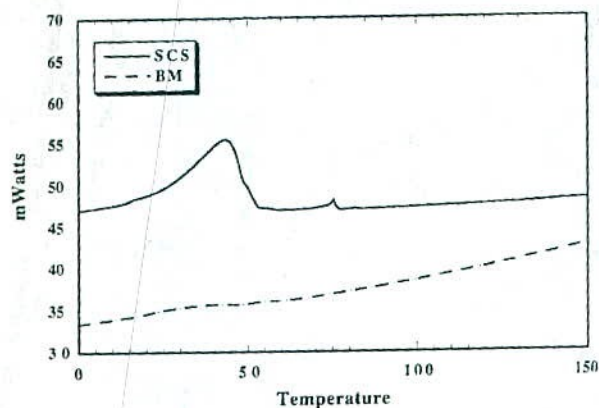


Figure 9. Differential scanning calorimetry thermograms of Bay Marchand and South China Sea wax from 0 to 100 °C.

molecular weight is, the lower the start of the melting range.³⁶ This would account for the slight bulge in the lower temperature regions for these five waxes. This further indicated that these five waxes had high molecular weights, branches, and rings. Eicosane was run as a standard to verify the accuracy of the melting enthalpies.

The remaining waxes all had distinct melting peaks as for SCS in Figure 9. MI was the only wax to have a definite transition peak and that merged into the melting peak. All had peak melting temperatures between 30 and 57 °C and melted over a range of 20-25 °C. Most had melting enthalpies between 10 and 35 cal/g. GM, Maya, AH, and CS had relatively low melting enthalpies, around 10 cal/g.

The melting temperatures of these paraffinic waxes were consistent with melting temperatures of waxes determined elsewhere. Several had melting temperatures very close to those found here, between 40 and 60 °C^{23,25,36} while some went as high as 93 °C, but none were lower than 40 °C.^{1,5,23} Other researchers' heats of fusion were near those determined here. Most were

(35) Wesolowski, M. *Thermochim. Acta* **1981**, *46*, 21-45.

(36) Giavarini, C.; Pochetti, F. *J. Therm. Anal.* **1973**, *5*, 83-94.

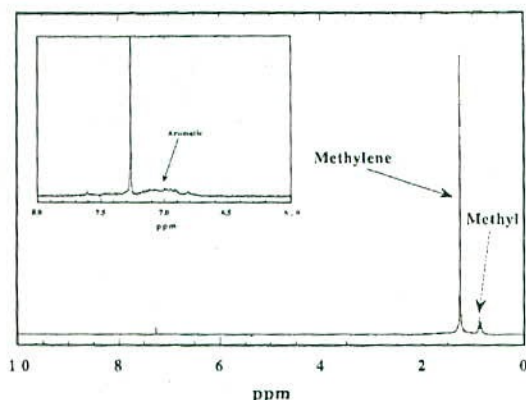


Figure 10. Arab Heavy in chloroform ^1H NMR spectrum at 5 g/L, with aromatic region expanded in inset.

Table 5. Percent Hydrogen for All Waxes Obtained by HNMR

crude	ratio CH_2/CH_3	acid H	ketone/ benzylic H	alcohol H	aromatic H
MI	7.22	0.11	0.15	0.01	0.23
SCS	4.47	0.28	0.35	0.00	0.44
B6	4.83	0.18	0.36	0.02	0.45
AB	5.10	0.26	0.38	0.00	0.50
ANS	4.89	0.15	0.27	0.00	0.46
SF	4.51	0.19	0.20	0.00	0.34
B4	4.90	0.17	0.33	0.02	0.52
Maya	4.57	0.15	0.29	0.00	0.52
AH	4.43	0.25	0.43	0.02	0.63
CS	4.63	0.28	0.40	0.01	0.54
GM	4.10	0.20	0.21	0.00	0.43
Alba	4.37	0.19	0.30	0.01	0.52
BM	3.25	0.27	0.32	0.00	0.55
T1	2.89	0.32	0.50	0.00	0.62
T2	2.45	0.29	0.38	0.00	0.53
SJV	2.47	0.28	0.31	0.00	0.65

between 18 and 34 cal/g^{25,37,38} with some as high as 50–60 cal/g.^{1,25}

Proton Nuclear Magnetic Resonance Spectroscopy. Proton NMR did not show as obvious a separation of the waxes as did the previous methods. A sample ^1H NMR spectra is shown in Figure 10. SJV, Alba, T1, T2, and BM did have a much lower ratio of methylene to methyl carbons as shown in Table 5. This implied a number of branches larger than those the other waxes, validating the DSC and FTIR data. The proton NMR spectra did show a small region of aromatic proton as in the inset in Figure 10, but none contained more than 1% aromatic. One percent aromatic carbon means that 7% of the wax contains an aromatic ring. Much of this aromatic carbon could be present in thiophenic rings as the sulfur present in the waxes was likely thiophenic. Also, among the paraffin waxes, an increase in sulfur content is accompanied by a corresponding decrease in H/C ratio. If the sulfur was thiophenic, then the aromatic carbons in the thiophene would serve to reduce the H/C ratio as was observed.

All the waxes contained near 1% aromatic carbon, not completely accounting for the decrease in the H/C ratio. For example, for a H/C ratio of 1.85, a $\text{C}_{28}\text{H}_{52}$ formula is required. This has over 14% CH. Since the wax has only an average of 1% aromatic carbon, most of the

Table 6. Percent Carbons from ^{13}C NMR for Several Waxes

crude	CH_2/CH_3 ratio for straight CH_2	% unknown	methyl branch (19.7 ppm)	methine for methyl branch (32.8 ppm)
MI	17.20	18	0.0	0.4
SCS	17.69	29	1.4	0.6
B6	16.78	19	2.0	1.5
AB	14.75	30	1.4	1.1
ANS	13.90	35	0.6	0.9
SF	10.24	36	1.7	1.2
B4	16.38	33	1.6	1.1
Maya	12.39	32	1.6	1.3
AH	21.10	37	1.1	0.7
CS	15.38	49	1.1	0.9
GM	9.85	47	1.2	1.2
Alba	15.33	42	1.9	1.3
BM	11.90	55	1.6	1.8
T1	19.91	31	2.0	1.5
T2	11.13	45	4.5	2.9
SJV	9.71	64	2.3	1.6

decrease must be attributable to naphthenic rings. Two rings would give 10% methine with a H/C ratio of 1.96. Since the methine peak is hidden in the methylene peak, this is probably what occurs. One group of researchers found aromatic contents of 0.3–4.6% aromatic carbon for wax fractions from petroleum.³⁹

The ^1H NMR spectra also indicated the type of oxygen present in the waxes. Most protons on carbons adjacent to alcohols or ethers absorb between 3 and 4 ppm. Less than 0.02% proton was located there, so virtually none of the oxygen was alcohol or ether. A peak at 2.3 ppm often occurred when protons were on a carbon adjacent to an acid group. Another peak at 2.55 ppm could be attributed to protons adjacent to a ketone or on a carbon adjacent to an aromatic ring. All of these peaks were less than 1% of the hydrogen in the molecule. Both methine and naphthenic peaks were obscured by the methylene peak.

^{13}C Carbon Nuclear Magnetic Resonance Spectroscopy. Carbon NMR has often been a useful tool in the characterization of compounds though it is less useful for petroleum wax because of the wax's heterogeneity. In ^{13}C NMR, the position of carbons four atoms away from the one being studied can affect the peak location. Thus peaks only exist where a large fraction of the wax molecules have the same characteristics.

Since most waxes contain long straight chains, eicosane was used to identify some of the peaks. The peak at 14.1 ppm was the methyl end of the chain. The peaks at 22.7, 31.9, and 29.1 ppm were methylene units successively further from the methyl group. The large peak at 29.7 ppm was long internal methylene.^{40,41} The ratio of these methylene to the 14.1 ppm methyl peak is tabulated in Table 6. This is an indication of the average length of a straight methylene chain terminated by a methyl group. Sample spectra are shown in Figure 11. The MI, B6, AB, ANS, SF, and SCS wax spectra were almost exactly like the spectra of eicosane. The spectra for AH, CS, GM, Maya, and B4 were much the same with a few additional small peaks. The peak at

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(40) Randall, J. C. *J. Polym. Sci. Polym. Phys. Ed.* **1973**, *11*, 275–287.

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(38) Srivastava, S. P.; Tandon, R. S.; Verma, P. S.; Pandey, D. C.; Goyal, S. K. *Fuel* **1995**, *74* (6), 928–931.

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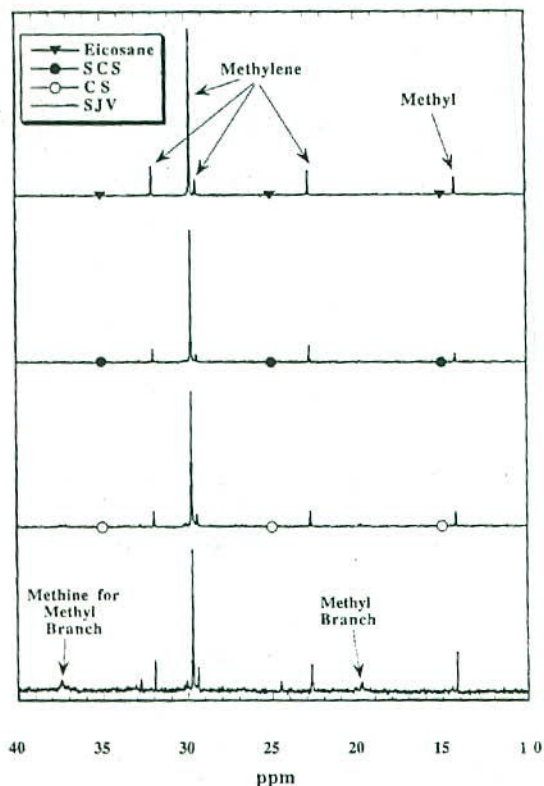


Figure 11. ^{13}C NMR for eicosane, South China Sea (SCS), Canadon Seco (CS), and San Joaquin Valley (SJV) waxes.

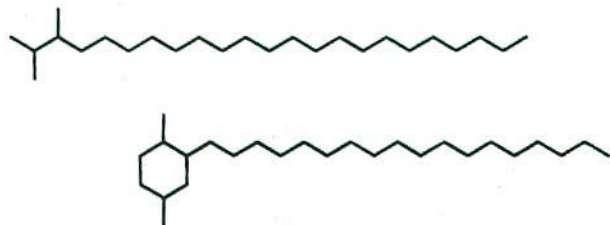


Figure 12. Average structure of a paraffin wax—Malu Isan.

19.71 ppm is indicative of methyl branches on a straight methylene backbone, and the peak at 32.8 ppm is the methine associated with that methyl branch. The percent of each of these peaks is shown in Table 6. The other waxes, SJV, BM, Alba, T1, and T2, also exhibited such peaks, as well as several unknown peaks. These waxes showed significantly more clutter under the baseline as tabulated in Table 5 as "% unknown". This was likely the result of a number of peaks overlapping. Thus, these waxes were more heterogeneous than the others.

None of the waxes exhibited any aromatic, ketone, or acid peaks. This was not surprising considering the high concentration of an identical region necessary for a peak to be formed. Given the high number of possible neighborhoods for any of the groups, the possibility of having enough of any to show in a spectra is unlikely.

Average Structure of Wax Molecules. Several of the data sets, when combined, were used to produce an average structure for each of the waxes (see Figures 12 and 13). From the data, three independent equations could be written for an overall wax molecule. Because four independent variables exist, one of the variables must be fixed. Quaternary carbon was chosen because

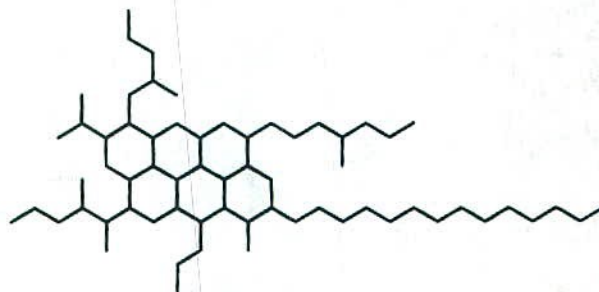


Figure 13. Average structure of a microcrystalline wax—San Joaquin Valley.

Table 7. Percent Methyl, Methylene, Methine, and Quaternary Carbon for All Waxes

crude	% methyl	% methylene	% methine	% quaternary carbon
MI	12.2	82.3	5.3	0.2
SCS	16.4	68.3	15.1	0.3
B6	15.1	68.2	16.5	0.3
AB	14.9	70.7	14.1	0.3
ANS	14.9	71.1	13.7	0.2
SF	15.9	66.7	17.2	0.2
B4	14.7	67.1	17.9	0.3
Maya	15.3	65.2	19.3	0.3
AH	15.8	65.2	18.7	0.4
CS	15.1	65.3	19.2	0.4
GM	16.8	64.2	18.7	0.3
Alba	15.3	62.3	22.1	0.3
BM	17.6	53.1	28.9	0.3
T1	19.3	52.1	28.2	0.4
T2	20.7	47.3	31.7	0.3
SJV	20.6	47.7	31.3	0.4

of its relative scarcity. The percent quaternary carbon was determined from the aromatic and acidic proton percentages from ^1H NMR. All of the aromatic proton was assumed present in benzylic rings. The acidic carbons and the benzene ring joints were assumed to be the only quaternary carbon present in the wax molecule. The three equations used to calculate methyl, methylene, and methine percentages are shown below

$$\#C + \#CH + \#CH_2 + \#CH_3 = \#C_{\text{total}} \quad (1)$$

$$\#CH + 2\#CH_2 + 3\#CH_3 = \#H_{\text{total}} \quad (2)$$

$$\frac{\text{CH}_2}{\text{CH}_3} = A \quad (3)$$

where A is the methyl-to-methylene ratio from ^1H NMR. The three equations were solved, and the numbers converted to percentages. These percentages are shown in Table 7.

For all of the waxes, the estimated percent quaternary carbon was below 0.5 wt %. The waxes averaged 12–21% methyl, 48–82% methylene, and 5–31% methine. Discrepancies between these data and the methylene content from FTIR was attributable to uncertainty in the data used to determine the percents methyl, methylene, and methine carbon. MI, SCS, B6, AB, ANS, and SF had, overall, the lowest percent methine, and methyl and the highest percent methylene. The extremely high percent methine of Alba, BM, T1, T2, and SJV combined with the percent methyl indicated a large number of rings. For every methyl group, there must be a corresponding methine, excepting the two terminal methyls present in any ringless molecule. The additional me-

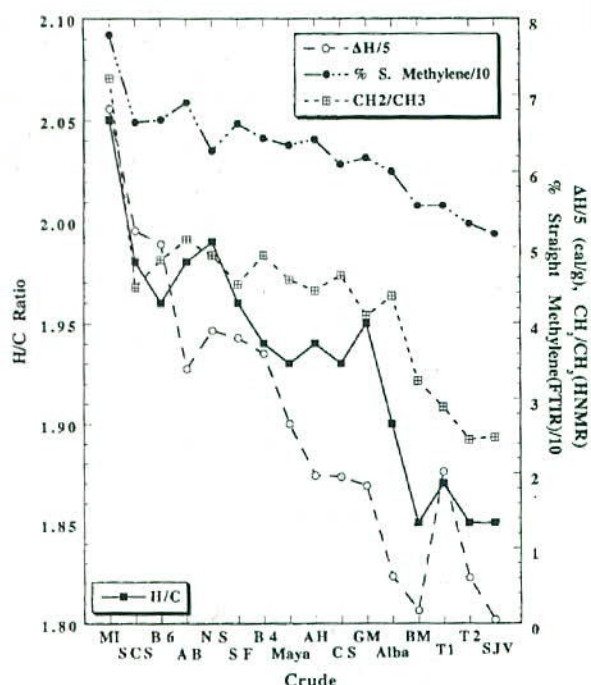


Figure 14. Comparison of ΔH , CH_2/CH_3 (^1H NMR) ratio, H/C ratio, and % straight chain methylene from FTIR for waxes from a variety of crudes.

thine above the number required for the methyl groups are ring joints. These five waxes had 7–11% more methine than methyl. This indicated a significant number of rings. MI, SCS, B6, AB, ANS, and SF, however, had about as many methine as methyl. This indicates that these waxes contained 0–2 rings per molecule.

Comparison of Different Techniques. BM, Alba, SJV, T1, and T2 were identified by several techniques as being different from other waxes. They had a large molecular weight range and lower H/C ratios. They had broad, indefinite melting regions. They had a smaller number of methylene groups per methyl than the other waxes, were a different color, and behaved much like gels. They had a lower percentage of straight chain methylene and a significant amount of unknown material in the ^{13}C NMR. They had a higher percent methine than methyl groups. These waxes were different from the others in a variety of ways and were thus a different type of wax called microcrystalline or amorphous wax.⁴² AB, NS, B6, MI, SCS, and SF had very different characteristics from the previous group. They had a narrow molecular weight range, between 300 and 650 amu. They had higher H/C ratios and identifiable melting regions. They had a higher number of methylene groups per methyl and were solids. They had a higher percentage of straight chain methylene. They had the near the same percent of methyl groups as methine, or more. These were labeled paraffin waxes. Five crude waxes did not fit neatly into either category. B4, AH, Maya, GM, and CS were mixtures of both types of wax with paraffinic wax at low molecular weight but a distinct tail of higher molecular weight compounds. A comparison of all these characteristics is shown in Figure 14. The mixed waxes tend to be high

in some respects but low in others because they are mixtures. In fact, most of the waxes were probably mixtures of both types, but the majority had one type dominating to such a degree that its characteristics almost completely obscured the other type.

Conclusions

The wax from sixteen crude oils was characterized. All of the waxes contained small amounts of oxygen which ^1H NMR indicated was primarily acid or ketone. All had small amounts (~1%) of aromatic carbon, a significant portion of which was probably involved in thiophenic rings. All contained at least 50% straight chain methylene.

The waxes fell into two different categories based primarily on molecular weight, H/C ratio, and melting ranges. Several of the waxes were composed of paraffinic hydrocarbons such as *n*-alkanes and isoalkanes. Their molecular weight range was between 350 and 600 amu with H/C ratios between 1.92 and 2.05. They displayed definite melting peaks and heats of fusion. These were paraffin waxes. Five other waxes, SJV, Alba, BM, T1, and T2 had a much longer molecular weight range continuing to 3600. These waxes also had slightly lower H/C ratios, between 1.85 and 1.90, with no identifiable melting peak. They had a much lower ratio of methylene to methyl carbons and contained a number of methyl branches. They had a large amount of clutter in their ^{13}C NMR spectra. Analysis indicated a large excess of methine compared to methyl groups. These were microcrystalline waxes.⁴² B4, AH, Maya, GM, and CS were a mixture of both types of wax, with the majority being paraffinic but with a significant amount of microcrystalline as well.

Precipitation by itself was not a viable method for the measurement of the wax content of a crude because material often coprecipitated with it. Two different types of wax were found in crude oil. They had differing H/C ratios, DSC plots, mass spectra, CH_2/CH_3 ratios from ^1H NMR, and ^{13}C NMR spectra.

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Nomenclature

- #C = number of quaternary carbon in wax molecule
- #CH = number of methine groups in wax molecule
- #CH₂ = number of methylene groups in wax molecule
- #CH₃ = number of methyl groups in wax molecule
- #C_{total} = total number of carbons in wax molecule (wt % C × given molecular weight)
- #H_{total} = total number of hydrogens in wax molecule (wt % H × given molecular weight)
- A = methylene/methyl ratio for wax from FTIR (Table 4)

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