

Señora Jefe
División de Nuevas Creaciones
Superintendencia de Industria y Comercio
E. S. D.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



Fecha de Recibido: 07/09/2011 Dep. 2011 NUEVAS CREACIONES
Tr. 1 PATENTACIÓN Tr. 075 FASE NACIONAL
Act. 03 PETICION Folios: 2

Referencia: Solicitud de Patente de Invención PCT (Fase Nacional)
Título: Capítulo I.
Expediente No. Bebida no alcohólica enriquecida con $^1\text{H}_2$ ^{16}O
Solicitante: 07-092.335
Asunto: Woodford Associates Limited
Pago del examen de patentabilidad.

MONICA WOLF WASSERMAN, identificada con cédula de ciudadanía No. 51'711.267 de Bogotá y con Tarjeta Profesional de Abogado No. 46.034 del Consejo Superior de la Judicatura, como apoderada de la sociedad, WOODFORD ASSOCIATES LIMITED, Por medio de la presente me permito allegar el recibo de consignación que respalda el pago del examen de patentabilidad, Capítulo I, para la Patente de Invención PCT (Fase Nacional), identificada en la referencia, a efectos de que ese Despacho proceda a la práctica del mismo.

ANEXOS

Recibo de consignación N°. 08-79.466 por la suma de cuatrocientos veinte mil pesos m/cte (\$420.000).

NOTIFICACIONES

Recibiré notificaciones en la Secretaría de ése Despacho y en mi oficina, ubicada en la calle 83A No. 23-90 de esta ciudad.

De la Señora Jefe,

MÓNICA WOLF WASSERMAN
C.C. No. 51.711.267 de Bogotá
T.P.A. 46.034 del C. S. de la J.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 8001760

RECIBO OFICIAL DE CAJA : 08 - 79,466
FECHA : OCTUBRE 6 DE 2008

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-092335-00005-0000

06/10/2008 10:08:16 20/08 Dep 2020 NULCRLACIONE
No 01 PATENTABILIDAD Lve 378 FASE NACIONAL
Act 538 PLATACION Folios 2

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
WOLF ABOGADOS ASOCIADOS	CONSIGNACION	BANCO DE BOGOTA	062754387	3460553	06/10/2008	4,053,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	85 EXAMEN DE PATENTABILIDAD DE INVEN	420,000.00
		TOTAL :	\$ 420,000.00

SON: CUATROCIENTOS VEINTE MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

DIVISIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 07-92335

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **591** DE

FECHA **30 DE ABRIL DE 2008** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **30 DE JULIO DE 2008**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____

NO X

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**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES**

2020

Bogotá, D. C., 14 de diciembre de 2010

Doctora

Mónica Wolf Wasserman

Representante

Woodford Associates Limited

ASUNTO

Radicación **07-92335**

Trámite 002

Evento 001

Actuación 430

Oficio

Folios **040**

Patente de Invención



Patente de Modelo de Utilidad



Vía Nacional



Vía PCT (fase nacional)



Cap. I



Cap. II



No. Sol. Internacional

PCT/RU2005/000045

Fecha de Presentación Internal.

8 de febrero de 2005

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

- 1.1. Descripción: Folios: 3 a 48 tal como se radicó inicialmente
- 1.2. Reivindicaciones: Folios: 49 a 52 tal como se radicó inicialmente
- 1.3. Dibujos: Folios: 54 tal como se radicó inicialmente
- 1.4. Prioridad: Ninguna

2. Objeto de la invención

- Título de la solicitud (folio 1)
Bebida no alcohólica enriquecida con $^1\text{H}_2^{16}\text{O}$
- Las reivindicaciones 1 a 14 de la solicitud en estudio se refieren a
 - 1.- Una bebida no alcohólica enriquecida con $^1\text{H}_2^{16}\text{O}$ en donde el contenido de $^1\text{H}_2^{16}\text{O}$ es no menos de 99.76% en peso de agua de dicha bebida no alcohólica, comprende:
 - A) un agua liviana de alta pureza en una cantidad entre cerca 20% a cerca de 99,9% en peso de dicha bebida no alcohólica, en donde el agua liviana altamente pura es una composición que comprende cerca de 99.76% a cerca de 99.99% de $^1\text{H}_2^{16}\text{O}$ y cantidades residuales de $^1\text{H}_2^{17}\text{O}$, $^1\text{H}_2^{18}\text{O}$, $^1\text{H}_2\text{H}^{16}\text{O}$, $^1\text{H}_2\text{H}^{17}\text{O}$, $^1\text{H}_2\text{H}^{18}\text{O}$, $^2\text{H}_2^{16}\text{O}$, $^2\text{H}_2^{17}\text{O}$, $^2\text{H}_2^{18}\text{O}$ hasta el 100% correspondientemente;

B) Un agua típica con un contenido típico de $^1\text{H}_2^{16}\text{O}$ en una cantidad entre 0% a cerca de 80% en peso de dicha bebida no alcohólica, en donde el agua es una composición que comprende entre cerca de 99.731% a cerca de 99.757% de $^1\text{H}_2^{16}\text{O}$ y cantidades residuales de $^1\text{H}_2^{17}\text{O}$, $^1\text{H}_2^{18}\text{O}$, $^1\text{H}^2\text{H}^{16}\text{O}$, $^1\text{H}^2\text{H}^{17}\text{O}$, $^1\text{H}^2\text{H}^{18}\text{O}$, $^2\text{H}_2^{16}\text{O}$, $^2\text{H}_2^{17}\text{O}$, $^2\text{H}_2^{18}\text{O}$ hasta completar el 100% correspondientemente; y C) un componente fisiológicamente aceptable en una cantidad de hasta 100% en peso de dicha bebida no alcohólica.

- El anterior producto busca proporcionar una bebida con más agua $^1\text{H}_2^{16}\text{O}$
- La Clasificación Internacional de Patentes (IPC) para esta solicitud es A 23 L 2/00

3. De la solicitud de Patente

3.1. Descripción (Artículo 28 D. 486)

La legislación es clara en establecer que la solicitud de patente deberá contener una descripción de la invención en términos que permitan la comprensión del problema técnico y de la solución aportada por la invención, exponiendo las diferencias y eventuales ventajas con respecto a la tecnología anterior.

El solicitante plantea que el agua liviana ($^1\text{H}_2^{16}\text{O}$) con un contenido de más de 99,76% es desconocido en la técnica (?) y que los métodos y aparatos para la producción de agua liviana son desconocidos en la técnica (?)

Además relaciona que la diferencia entre los isotopólogos, en cuanto a sus propiedades físicas, difieren apreciablemente (?) y que las moléculas que comprenden isótopos pesados en el organismo de los mamíferos puede llevar a un cambio de los procesos bioquímicos normales y a una disminución de los recursos funcionales del organismo, por lo que existe una sentida necesidad de incrementar el contenido de moléculas de agua liviana $^1\text{H}_2^{16}\text{O}$ y disminuir cualquier otra.

Luego el solicitante muestra "un método y un aparato para la producción de agua liviana altamente pura"

De lo anterior, esta oficina encuentra varias inconsistencias en la solicitud.

3.1.1. En relación al problema

Primero. Si bien es cierto que "las moléculas que comprenden isótopos pesados en el organismo de los mamíferos, puede llevar a un cambio de los procesos bioquímicos normales" estos solo sucedería si existiera un cambio radical en las características de las moléculas de agua. Durante miles de años los seres vivos que habitan el planeta tierra han empleado el agua con cantidades de la molécula $^1\text{H}_2^{16}\text{O}$ del orden del 99.731%¹ sin que se vea afectado su metabolismo o las funciones bioquímicas de estos seres vivos.

Segundo. No existe ninguna razón válida, ni en la descripción de la solicitud ni en literatura relacionada, para creer que el agua que existe en el mundo se esté modificando a un estado donde el deuterio se encuentre en mayor cantidad en el agua normal y mucho menos el tritio, el cual es mucho más inestable.

¹ Gat, Joel R. *Isotope hydrology*. Series in environmental science and management. Vol. 6 Pág. 10.

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Tercero. Muchos científicos han realizado estudios² de los efectos de reemplazar parte del protio por deuterio en agua, y crear agua pesada, pero los mismos científicos llaman a este tipo de agua "agua sintética" porque no es posible encontrarla de forma natural. Aún más, es deseable en algunos casos encontrar aguas con contenidos de deuterio más altos de lo normal para emplear como formas alternativas de obtener energía; pero en la naturaleza sólo se encuentra con algunas decimas de porcentaje por encima de lo normal.

Cuarto. El solicitante es ambiguo en la presentación de los problemas bioquímicos por usar agua pesada y sólo refiere que *puede llevar a un cambio de los procesos bioquímicos normales y a una disminución de los recursos funcionales del organismo*. La literatura refiere que el principal problema que han encontrado los científicos en el metabolismo de los seres vivos por reemplazar el protio por el deuterio, es que este último reacciona de la misma forma que el protio pero siete veces más lento. Sin embargo, también han encontrado, mediante experimentos con seres vivos en aguas pesadas, que después de un tiempo los seres vivos terminan adaptándose a su nueva situación³.

Quinto. Dado que el problema principal es la lentitud de la reacción del agua que contiene deuterio, en las proporciones que habitualmente se encuentra dicho deuterio en el agua⁴ es improbable que cause un trastorno fuerte en el metabolismo de un ser vivo, aún si la proporción de la moléculas que no son $^1\text{H}_2^{16}\text{O}$ aumentaran a escalas de un dígito de porcentaje.

Por consiguiente, el problema que plantea el solicitante no está sustentado y podría considerarse a la luz de la literatura que es inexistente

3.1.2. El solicitante también plantea que *métodos y aparatos para la producción de agua liviana son desconocidos en la técnica*

Desde que en diciembre de 1931 Urey, Brickwedde y Murphy anunciaron el descubrimiento del Deuterio en the American Physical Society y se publicó dicho descubrimiento en 1932 en Physical Review, se han realizado cientos de experimentos para aislar el deuterio partiendo de agua pesada y obteniendo agua liviana. Si bien no son explícitos en perseguir directamente producción de agua liviana, este es un producto que se obtiene al buscar el deuterio. Por consiguiente la afirmación del solicitante no resulta ser cierta.

3.1.3. Sobre métodos y aparatos para la producción de agua liviana

El solicitante en realidad no está suministrando ningún método nuevo ni aparato alguno para la producción de agua liviana. El solicitante refiere **solamente los principios de un proceso convencional de destilación**. Es claro que existen diferencias (muy pequeñas)⁵ en los puntos de ebullición de las variedades de agua y que un técnico medio consideraría inmediatamente el proceso de destilación, pero dicho proceso no resulta tan sencillo de emplear debido precisamente a las pequeñísimas diferencias en las temperaturas de ebullición; por consiguiente, ese sí es un problema y el solicitante no está dando una solución al mismo.

² Lynden-Bell, Ruth y otros. *Water and life. The unique properties of H₂O*. CRC Press. Pág 163.

³ Ibíd. Pág 164.

⁴ Gat, Joel R. *Isotope hydrology*. Series in environmental science and management. Vol. 6 Pág. 10.

⁵ Ibíd. Pág 11.

Además de lo anterior, supuestamente el solicitante refiere un aparato para hacer dicha separación, pero nunca describe dicho aparato en la descripción y sólo hay un dibujo que mostraría dicho aparato.

3.1.4. Sobre el equilibrio entre las diferentes moléculas de agua

La literatura refiere que existe un equilibrio químico entre las diferentes especies de moléculas de agua⁶ y se encuentran constantes que relacionan dicho equilibrio. Dada esta consideración, la "solución" planteada por el solicitante podría no existir, debido a que en el caso que se retirara una porción de moléculas que no tuviera $^1\text{H}_2^{16}\text{O}$ la reacción química podría desplazarse en el sentido de restablecer el equilibrio químico forzando a la aparición nuevamente de las moléculas indeseables. Al respecto el solicitante no se pronuncia.

3.1.5. El producto no tiene relación con el supuesto problema técnico

Lo reivindicado en la solicitud está referido a un uso que el solicitante quiere darle al agua que él "encuentra". Pero de lo visto en la descripción, el objeto de la solicitud es cómo incrementar el contenido de moléculas de $^1\text{H}_2^{16}\text{O}$ y disminuir cualquier otra molécula en el agua que se toma una persona y no como usar dicha agua. La legislación es clara en no permitir la protección de usos.

3.1.6. El producto a proteger corresponde a agua normal.

De acuerdo a lo relacionado por el solicitante, el 99,76% del agua que él denomina natural es $^1\text{H}_2^{16}\text{O}$ y propone una que tenga más de ese porcentaje. En primer lugar, como ya se dijo, es muy probable que después de lograr aumentar dicho porcentaje la reacción química se desplace nuevamente hacia su condición de equilibrio y no se presente el objetivo alcanzado; luego lo que debería ser problema de estudio es cómo se logra desplazar la reacción hacia más arriba; consecuencia de lo anterior, es que el solicitante está protegiendo agua normalmente encontrada en cualquier parte del mundo. En segundo lugar, lo que dicta la literatura científica es que el valor 99,76% **es un promedio internacional**, por lo que sí existen aguas "naturales" con porcentajes mayores a 99,76%. Esto conllevaría a que el solicitante pretendería proteger un elemento que se encuentra en la naturaleza, algo explícitamente prohibido para protección mediante patentes.

Por lo anterior, la descripción no cumple con el requisito de claridad y/o divulgación completa que permita su comprensión y ejecución de acuerdo al art. 28 de la Decisión 486 y/o art. 22 del Tratado PCT

3.2. Reivindicaciones (Artículo 30 D. 486)

El capítulo reivindicatorio de la solicitud en estudio presenta los siguientes defectos:

3.2.1. Sobre las inconsistencias encontradas en el capítulo descriptivo

De acuerdo a lo analizado en el punto 3.1, dichas observaciones también son aplicables a este capítulo.

⁶ Ibíd. Pág 22 y ss.

3.2.2. Sobre otras inconsistencias

El solicitante en la descripción (folio 15) explica que ofrece un método y un aparato para la producción de agua liviana altamente pura, por que agua liviana es desconocida en la técnica (folio 7). Sin embargo, en la reivindicación principal no solo no protegen métodos para la producción de aguas livianas ni aparatos, sino que además se contradice en la reivindicación 3 (y en otras posteriores) donde refiere que el agua liviana puede ser agua potable, destilada, desionizada o producida por ósmosis inversa, dando a entender que son conocidas las aguas y que cualquier técnico empleando cualquiera de dichos procedimientos tradicionales puede obtener el agua más liviana. Luego el problema nada tiene que ver con la solución y, además, lo que presuntamente desarrolla el solicitante, él mismo lo refiere como conocido. Además de lo anterior, ninguna de las técnicas mencionadas está relacionada en la descripción por lo que dichas reivindicaciones no tendrían sustento.

El solicitante emplea términos que no corresponden con el arte y que generan confusión al lector. Por ejemplo, agua de "alta pureza" en el lenguaje técnico señala agua natural con contenidos bajos de sustancias ajenas a la molécula de agua; no indica que sea agua con exclusivamente $^1\text{H}_2^{16}\text{O}$.

3.2.3. Sobre ambigüedades

El solicitante emplea frases como "cerca de" que no hacen explícito lo que quiere que se le proteja. Por consiguiente deben eliminarse.

Por lo anterior, el capítulo reivindicatorio no cumple con el requisito de definición, claridad, concisión o sustento por la descripción de acuerdo al art. 30 de la Decisión 486 y/o art. 22 Tratado PCT.

4. Determinación del Estado de la Técnica

De acuerdo a lo observado, **NO** es posible realizar una correcta **Determinación del Estado de la Técnica y Evaluación de los requisitos de patentabilidad**. Solo cuando se haya corregido adecuadamente la solicitud y se haya orientado de forma tal que sea claro lo que quiere ser protegido, sólo en ese momento esta oficina podrá elaborar una determinación correcta del estado de la técnica y una evaluación correcta de los requisitos de patentabilidad.

5. Conclusión:

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de SESENTA días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente. Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

José Luis Salazar López
Directora de Nuevas Creaciones (E)

Concepto Técnico No. **4160**

Examinador Técnico **202003**

El anterior requerimiento fue notificado por fijación en lista, de fecha _____

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Announcement

New manuscript guidelines for the reporting of stable hydrogen, carbon, and oxygen isotope-ratio data

For 75 years the International Union of Pure and Applied Chemistry (IUPAC) has been promoting cooperation among chemists of member countries. The Commission on Atomic Weights and Isotopic Abundances is one of the commissions of IUPAC. The recommendations of the Commission on Atomic Weights and Isotopic Abundances are definitive. For example, the recommendations for values of atomic weights of the elements are used in trade and commerce between the member nations of IUPAC. The National Adhering Organizations of the U.S., the U.K., Canada, and Germany are, respectively, the National Academy of Sciences, the Royal Society, the National Research Council of Canada, and the Deutscher Zentrallausschuss für Chemie.

It has been brought to the attention of the commission that confusion exists in the reporting of stable hydrogen, carbon, and oxygen isotopic results on noncorresponding scales because the supply of PDB (Pee Dee belemnite) has been exhausted and because SMOW (Standard Mean Ocean Water) does not have a unique definition. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values are often reported to 0.01‰ relative to PDB and "correct" interpretation of data in oceanography, atmospheric sciences, and other fields may depend on accuracies of the order of 0.05‰. Because the supply of PDB has been exhausted and laboratories do not use the same reference material to establish their carbon and oxygen isotope-ratio scales, different laboratories report widely varying values for the same material. For example, $\delta^{18}\text{O}$ values of NBS 19 carbonate expressed relative to PDB from 30 laboratories worldwide ranged from -2.42‰ to -1.53‰ and $\delta^{13}\text{C}$ values from +1.75‰ to +2.33‰ (Gonfiantini 1984).

To eliminate confusion in the reporting of light stable isotopic ratios, the commission has recommended that the use of SMOW and PDB be discontinued and that isotopic abundances of oxygen-, carbon-, and hydrogen-bearing materials be reported relative to the reference water VSMOW (Vienna Standard Mean Ocean Water) and relative to VPDB (Vienna Pee Dee belemnite) through the use of the reference material NBS 19 carbonate. Furthermore, oxygen and hydrogen isotopic abundances of *all substances* should be reported on scales normalized using SLAP (Standard Light Antarctic Precipitation).

In accord with recommendations (IUPAC 1994; Coplen 1994) by the Commission on Atomic Weights and Isotopic Abundances of the International Union of Pure and Applied Chemistry, we request that authors discontinue reporting isotopic abundances relative to SMOW (Standard Mean Ocean Water) and PDB (Pee Dee belemnite). Henceforth, hydrogen isotopic ratios of all substances should be expressed relative to VSMOW (Vienna Standard Mean Ocean Water) on a normalized scale such that the $\delta^2\text{H}$ of SLAP (Standard Light Antarctic Precipitation) relative to VSMOW is -428‰, carbon isotopic ratios of all substances should be expressed relative to VPDB (Vienna Pee Dee belemnite) on a scale defined by adopting a $\delta^{13}\text{C}$ value of +1.95‰ for NBS 19 carbonate relative to VPDB, and oxygen isotopic ratios should be expressed relative to either VSMOW or VPDB on a normalized scale such that the $\delta^{18}\text{O}$ of SLAP is -55.5‰ relative to VSMOW. Values of isotopic fractionation factors should be indicated if isotopic abundances of a mineral or compound depend on such a factor.

References

- COPLEN, T. B. 1994. Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. *Pure Appl. Chem.* 66: 273-276.
GONFIANTINI, R. 1984. Advisory group meeting on stable isotope reference samples for geochemical and hydrological investigations. Rep. 1 AEA.
IUPAC. 1994. Atomic weights of the elements 1993. *Pure Appl. Chem.* 66: 2423-2444.

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US005855921A

United States Patent [19]
Somlyai

[11] **Patent Number:** 5,855,921
[45] **Date of Patent:** Jan. 5, 1999

[54] **PHARMACEUTICAL PRODUCTS FOR CURING TUMOROUS DISEASES AND PROCESS FOR PREPARING SAME**

4,411,798 10/1983 Chan 210/727
4,444,737 4/1984 Tsuchiya et al. 423/249
5,223,269 6/1993 Liepins 424/600

[76] **Inventor:** Gábor Somlyai, 51/a, Deák F. u., Budapest 1215, Hungary

OTHER PUBLICATIONS

[21] **Appl. No.:** 211,941

The Merck Index, 10th Ed (1983) pp. 424-425.

[22] **PCT Filed:** Sep. 28, 1992

Remingtons Pharmaceutical Sciences, 16th Ed., Arthur Osol, Editor in Chief (Jan. 1980) pp. 1239-1243.

[86] **PCT No.:** PCT/HU92/00036

Internal Medicine, 4th Ed. Editor-in-Chief Jay Stein, pp. 699-715 (Jan. 1993).

§ 371 Date: Jun. 2, 1994

§ 102(e) Date: Jun. 2, 1994

[87] **PCT Pub. No.:** WO93/08794

PCT Pub. Date: May 13, 1993

Primary Examiner—Brian M. Burn

Attorney, Agent, or Firm—Gordon-Lendvay & Catan

[30] **Foreign Application Priority Data**

[57] **ABSTRACT**

Oct. 31, 1991 [HU] Hungary 3437/91

The invention relates to products for curing tumorous diseases, comprising as active agent water or aqueous solutions, suitable for human consumption, having a deuterium content of 0.1 to 110 ppm, optionally together with carriers and/or auxiliaries, in the form of pharmaceutical products or medicinal solutions. Furthermore, the invention relates to a process for preparing these products.

[51] **Int. Cl.⁵** A61K 9/08

[52] **U.S. Cl.** 424/600; 423/580.2; 423/647.7

[58] **Field of Search** 424/600; 423/580.2, 423/647.7

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,807,960 4/1974 Thayer 23/270 R

34 Claims, 2 Drawing Sheets

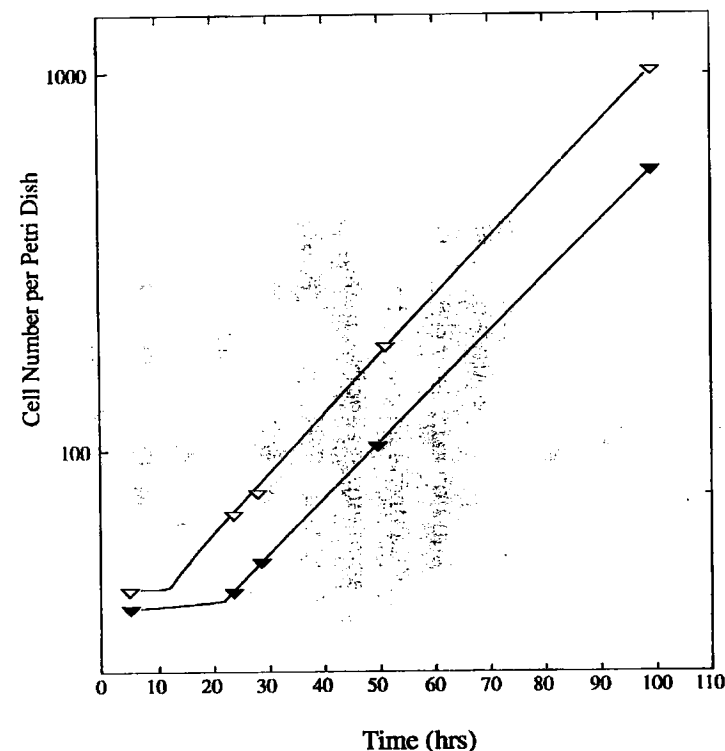
U.S. Patent

Jan. 5, 1999

Sheet 1 of 2

5,855,921

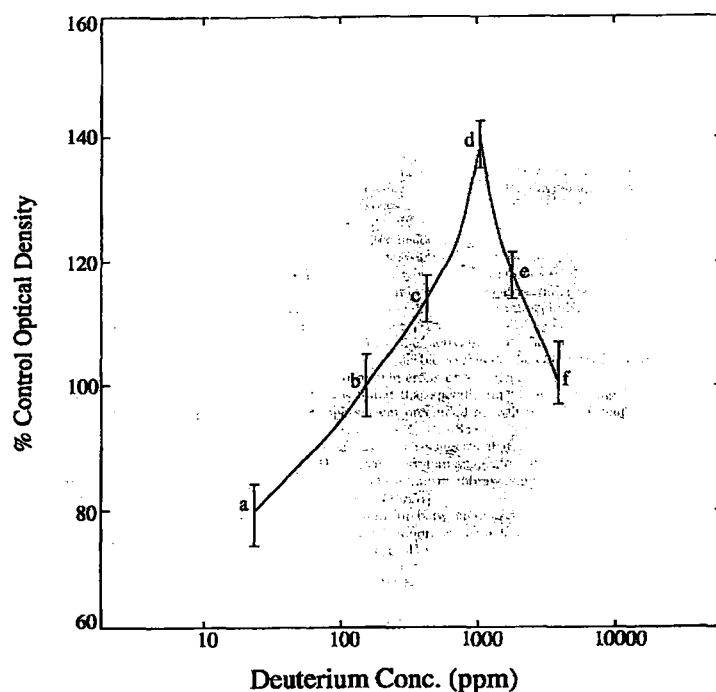
Fig. 1



Ojo... Antecedente clave

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Fig. 2



1 PHARMACEUTICAL PRODUCTS FOR CURING TUMOROUS DISEASES AND PROCESS FOR PREPARING SAME

This invention relates to pharmaceutical products for curing tumorous diseases and a process or preparing same.

Nowadays there are a number of methods (surgical intervention, therapy by irradiation, hormone therapy, applying antitumor agents) for combating against malignant tumors. All these methods together with the recent results in diagnostics have resulted in major advances in the last decade, but in spite of these results the applied methods have many disadvantages.

The main reason of the problem is that since the molecular mechanism of the cell-proliferation is still not known, the intervention into the course of the disease by the available means is rather difficult. It is therefore that the way of the recovery or at least a delay in the development of the disease involve frequently the removal of some parts of organs or troubles in the blood-forming process when antitumor agents, etc. are used.

The proper answer to this question can be expected only with full knowledge of the (sub) molecular processes playing a key role in initiating the cell proliferation.

By means of the development of the molecular biology, biological science is just before recognizing the decisive regulating processes through which it will be possible to restore patients suffering from tumorous diseases to health.

Taking into consideration the recent results of molecular biology the conclusion can be drawn that a common early response of eukaryotic cells to stimuli which activate the proliferation is an increase in intracellular pH due to an exchange of extracellular Na^+ for intracellular H^+ across the plasma membrane [P.N.A.S. 79, 7778-7782 (1982)]. The conclusion that the activation of the Na^+/H^+ exchange system is indispensable to initiate the cell growth has been supported by numerous experimental results.

It was found that specific mutation abolishing Na^+/H^+ exchange system precluded growth of cells at neutral and acidic pH [P.N.A.S. 81, 4833-4837 (1984)].

Recent evidence suggests that growth factors activate Na^+/H^+ exchange system suggesting that Na^+/H^+ exchange may function as a transmembrane signal transducer [Nature 304, 645-648 (1983)].

The relationship between the activated Na^+/H^+ exchange system and the tumorous character of cell lines have been proved, too. The pH was found to be increased after transformation of cell line comparing to the nontumorigenic parental line [(P.N.A.S. 84, 2766-2770 (1987)). Strong correlation was found between the expression of oncogene and the elevated intracellular pH, because injecting the protein encoded by Ha-ras or the expression of V-src and Ha-ras oncogenes in the cell increased the intracellular pH by activating the Na^+/H^+ exchange system [Mol. Cell. Biol. 7, 1984-1988 (1987); Gene 54, 147-153 (1987)].

Similar changes were found by activating another membrane-bound hydrogen-ion transport system. In this case an ATPase gene was isolated from yeast, and used to transform mouse and monkey cell lines. The gene was expressed and the ATPase, by continually extruding protons, caused a sustained alkalization of the cytoplasm. The really surprising result of this experiment was that the cells transformed by the gene of ATPase of the yeast acquired tumorigenic properties [Nature 334, 438-440 (1988)].

This latest experiment proves that the induction of cell proliferation is connected not only with the activation of the Na^+/H^+ exchange system, but also with the activation of an

other proton-extruding system which can serve as a signal to cell proliferation, in general.

A simple explanation for the above phenomenon can be that the cell proliferation is initiated by increase in intracellular pH. This explanation was, however, not confirmed by the experiments in which an artificial increase in cytoplasmic pH has not raised the cell proliferation activity [J. Exp. Biol. 124, 359-373 (1986)].

The above-mentioned molecular processes can be interpreted by investigating the possible role of hydrogen and of deuterium in controlling the above processes.

In nature the ratio of hydrogen to deuterium is about 6000:1. Because of the mass difference of 100% the two isotopes show different behaviour in chemical reactions. It is a generally accepted view that the D-bonds participating in chemical reactions can be split at a lower rate because of the isotope effect, therefore they need an augmented activation energy [Simonyi Miklós and Fitos Ilona: Hydrogen Isotope Effect in Chemical Reactions. A kémia újabb eredményei (in Hungarian). (Recent Results in Chemistry) 46, 8-129 (1980)]. In enzymatic reactions it can be similarly measured that the reaction rate is of from 4 to 5 times higher with the hydrogen isotope having the smaller mass number [Biochem. Pharmacol. 30, 3089-3094 (1981)].

The effects of the deuterium have been thoroughly investigated in biological systems, too [Katz, J. J. and Crespi, H. L.: Isotope Effects in Biological Systems (eds. Collins, C. J. and Bowman, N. S.) A.C.S. Monograph 167, van Nostrand Reinhold, New York, 1971, 286-363]. The common characteristic of these experiments is that the investigation of the deuterium effects is usually carried out by applying high (1-100%) D_2O concentrations.

It is widely accepted that the deuterium has an inhibiting activity to the reproduction and growth of bacteria, yeasts and plants. Mammalia can tolerate the D_2O in a concentration of at most 35%; a higher concentration of D_2O has a lethal effect to them.

In these experiments the deuterium was applied in from 100 to 10,000 times higher concentrations than the natural concentration which was ignored.

A world-wide survey of hydrogen isotopes in precipitations revealed that the D content covers a range of 120-160 ppm depending mainly on the site of sampling. Plants and thus also algae differentiating between hydrogen isotopes are able to enrich hydrogen [Schiegl, W. E. and Vogel, J. C., Earth and Planet. Sci. Letters 7, 307-313 (1970); Ziegler, H. et al., Planta 128, 85-92 (1976)]. As a result of these processes the deuterium concentrations e. g. in plant-eating creatures show alterations in a narrow range, depending on the species and quantities of the plants consumed. In case of humans the area, where the consumed plants were cultivated, is a decisive factor. According to measurements the deuterium content of the rainfall on the tropics is 155-160 ppm, whereas this value is only 120-150 ppm in the temperate zones of the world. The difference becomes visible in the deuterium content of the plants, too, with alterations of up to 10-20 %.

Though the above-mentioned phenomena have been observed, the experts have not attached any importance to the deuterium content in biological systems.

The aim to be achieved by the invention was to develop pharmaceutical products suitable for preventing cancer or inhibiting the tumor growth and, in this way, curing cancer diseases.

The basis of the invention is the recognition that the deuterium content of very low level (120-160 ppm) in biological systems is essential for maintaining the normal

rate of cell proliferation and the deficiency of deuterium increases the length of cell cycles. It was namely realized that the deuterium is a component of a submolecular regulating system and the processes temporarily elevating the concentration of D trigger cell proliferation.

A further basis of the invention is the recognition that by applying water or aqueous solutions containing deuterium in an amount less than the deuterium content of natural waters (e.g. juices diluted with deuterium-depleted water), the deuterium level in the human organism can be decreased as a result of exchange processes and in this way the proliferation of tumorigenic cells can be stopped or the development of cancerous tumors can be prevented.

Consequently, the invention relates to products for curing tumorous diseases, comprising water with a deuterium content of 0.1 to 110 ppm and/or aqueous solutions, suitable for human consumption, with a deuterium content of 0.1 to 110 ppm as active agent, optionally together with carriers and/or auxiliaries.

The products according to the invention are preferably pharmaceutical products like physiological salt solutions, or medicinal solutions like fruit syrups, soft drinks, or beer with reduced alcohol content or free from alcohol, having a deuterium content of 0.1 to 110 ppm.

Furthermore, the invention relates to a process for preparing products for curing tumorous diseases, comprising the steps of producing by electrolysis and/or distillation water and/or aqueous solutions having a deuterium content of 0.1 to 110 ppm as the active agent, then transforming the thus-produced water and/or aqueous solution having a deuterium content of 0.1 to 110 ppm optionally together with carriers and auxiliaries to pharmaceutical products or medicinal solutions.

According to a preferred method of execution of the claimed process physiological salt solutions are prepared as pharmaceutical products having a deuterium content of 0.1 to 110 ppm.

According to a further preferable method of execution of the process of the invention fruit syrups, soft drinks, or beer with no or reduced alcohol content are prepared as medicinal solutions having a deuterium content of 0.1 to 110 ppm.

The products according to the invention are preferably formulated as injectable solutions, infusion solutions, syrups, juices or hydrating ointments having a deuterium content of 0.1 to 110 ppm.

The products according to the invention are suitable for curing tumorous diseases. The basis of this therapeutic application is the fact that by applying deuterium-depleted solutions containing deuterium from 0.1 to 110 ppm the deuterium level of the organism decreases, too. In consequence of this process the growth rate of the tumorous cells is slowing, then these cells will decay, while the healthy cells are still capable of tolerating decreasing deuterium concentration.

The suitability of the process according to the invention for treating tumorous diseases has been proved by in vitro and in vivo tests carried out by using deuterium-depleted water. The test results can be seen in FIGS. 1 and 2 as well as in Tables 1 to 4.

BRIEF DESCRIPTION OF THE DRAWINGS

In FIG. 1 the growth of mouse-fibroblast cells L₂₂₉ is illustrated after synchronization in phase G1 in nutrient fluids prepared by using water having decreased (v: 30 ppm) or normal (v: 150) deuterium content.

In FIG. 2 the results obtained at determining the relative amount of mouse-fibroblast cells L₂₂₉ is illustrated after

cultivating them in nutrient fluid media containing deuterium in an amount of 30 to 5000 ppm (a: 30; b: 150; c: 300; d: 600; e: 1250; f: 5000 ppm D).

When the hydrogen obtained by electrolyzing water was burned to water according to Example 1, water containing deuterium in an amount of 30-40 ppm was produced. Water containing deuterium in higher concentrations than natural waters was prepared by adding D₂O of 99.78% by mass to normal water. Culture media suitable for maintaining in vitro different animal cell lines were produced of the so-prepared waters having different deuterium contents by dissolving 10 g of a commercially-produced dehydrated mixture of amino acids, vitamins, salts and bases [Dulbecco's modified Eagle's medium (D'MEM), code number: 074-01600; Sigma, St. Louis, USA] in 1 litre of water, 110 ml of bovine serum were added to the so-prepared solutions. The so-obtained liquid medium contained all the compounds needed for maintaining cells.

The growth of mouse-fibroblast cells L₂₂₉ was first studied under in vitro conditions in nutrient media containing deuterium in different amounts (30-5000 ppm). By these experiments the division of about 400 individual cells was followed. The experiments proved that the cell growth rate in a medium prepared with D-depleted water was reduced by 15-20%.

Then it was investigated whether the deuterium concentration of the nutrient media influenced the length of time necessary for the cell to enter into S-phase from the so-called G1 phase after synchronization (FIG. 1). It can be seen in FIG. 1 that, after synchronizing, the growth of the cells started 6-8 hours later and the growth rate was lower in nutrient media prepared with D-depleted water (v: 30 ppm) than in water of normal deuterium level (v: 150 ppm).

A method generally accepted in the recent years for determining the number of cells is carried out by incubating the cells together with 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium-hydroxide (XTT) which compound is reduced by the cells. The reduced form of this compound shows an absorption maximum at 450 nm and thereby its amount can be photometrically measured; thus, the relative cell number can be calculated from the optical density (OD) values [Cancer Research 48, 4827-4833 (1988)]. By this method the effects of deuterium concentrations lower (30 ppm) and higher (300-5000 ppm) than the natural level on cell proliferation were also studied (FIG. 2). It was proved by the experiments that the growth rate was slowing down in nutrient media prepared with deuterium-depleted water. According to these experiments deuterium concentrations from 2 to 4 times higher than the natural one (300 and 600 ppm) exert a stimulating effect on cell-proliferation. [Increasing further the deuterium concentration (1250 and 5000 ppm) the inhibition deriving from the isotope effect will become dominant.] Having repeated the tests with four further different cell lines similar results were obtained.

In the first in vivo experiment it was investigated how the growth of the tumors was influenced by decreasing the deuterium content in the drinking water of mice. Human breast tumours MDA-MB-231 and MCF-7 were transplanted into two groups of 14 CBA/Ca mice each. The animals of the control group consumed normal water, while from the day following the transplantation the animals belonging to the treated groups were supplied with D-depleted water prepared according to the invention. The results are summarized in Table 1.

TABLE 1

Effect of deuterium-depleted water on the growth of breast tumour in mice

Cell line	MDA-MB-231		MCF-7	
	Control	Treated	Control	Treated
Days				
20	5/5	9/9	6/6	8/8
30	5/5	5/9	5/6	5/8
40	1/1	4/8	2/2	4/7
71	0/0	3/8	1/2	2/7
80	0/0	2/7	0/1	0/5
87	0/0	1/6	0/1	0/5

The numbers in the treated and control groups correspond to the numbers of tumorous/all animals.

The results in Table 1 show that spontaneous tumour regression could be experienced only in one animal of the 11 tumorous animals in the two control groups (5+6), the others perished on the 71th and 80th day, resp., after transplantation. In the two treated groups, on the contrary, the tumor, after having grown, regressed and then disappeared in 10 animals (59%) of the 17 tumorous animals (9+8) and one of the tumorous animals, which is not shown in Table 1, survived by 30 days the animal which died at the latest in the control group. These animals got drinking water containing 30 ppm of deuterium for 3 weeks, then water containing 110-120 ppm of deuterium to the end of the experiment.

Carrying out an other experiment tumorous human prostatic cells PC-3 were transplanted into mice of 44 CBA/Ca type. The treatment started on the 32nd day after transplantation by giving the animals water containing 94±5 ppm of deuterium to drink. At this time the average tumour diameter was 10.4 mm and 10.2 mm in the control and the treated groups, respectively. (Taking into consideration the body/tumour mass ratio, the treatment of the mice at this stage of the experiments corresponds to starting the cure of a human being of 70 kg body mass with a tumour of 3.5 kg.) Thus, the treatment was started in a very advanced stage of tumor, and that was the reason why the treatment was not as effective as in the first experiment. The numbers in Table 2 represent the number of the tumorous/all animals and the average tumor size changing in the of the treatment.

TABLE 2

Effect of deuterium-depleted water on PC-3 prostatic tumor in mice

Days	Tumorous/all animals		Average tumour diameter (mm)	
	Control	Treated	Control	Treated
32	22/22	22/22	10.4	10.2
39	17/17	19/20	14.6	11.1
46	13/13	17/19	22.4	17.5
53	9/9	11/14	21.7	15.0
60	6/7	10/13	23.8	15.0
67	3/4	10/13	16.7	18.0
74	3/4	7/10	24.0	18.1
81	3/4	5/8	28.6	18.0
88	2/3	5/8	37.0	16.6

The results in Table 2 show that in the control group only 3 animals (13%) out of 22 survived the 87th day after transplantation of the tumorous cells, while in the treated group 8 animals (36%) out of 22 were still alive on the 88th day. At this time two animals (9%) and five animals (23%), resp., were alive in the control and the treated groups, respectively. In 3 animals of the treated group tumor regression could be observed. A significant difference between the

treated and the control groups, resp., is confirmed by the data of the average tumor diameter, too.

On the basis of the data in Table 2 cumulative mortality data are summarized in Table 3.

TABLE 3

Cumulative mortality data on PC-3 prostatic tumor

Days	Control	Treated
32	0	0
39	5	2
46	9	3
53	13	8
60	15	9
67	18	9
74	18	12
81	18	14
88	19	14

The results in Table 3 show that the number of animals perished in the treated group was smaller than that of the control group in all stages of the test. It is worth accentuating that by the 67th day after transplantation only 9 animals perished in the treated group, while twice as much animals (18) perished in the control group. The importance of this difference is underlined by the fact that the tumor development period of one week in mice corresponds to a period of 200-300 days in humans. Thus the data in Table 3 show that the survival period of humans can be increased by years, even if the medical cure starts in an advanced stage of the disease.

In a third experiment tumorous HT-29 colon cells were transplanted into mice. The treatment by giving the animals water containing 94±5 ppm of deuterium to drink started on the 24th day after transplantation. The average tumor volumes can be seen in Table 4. The control group consisted of 13 animals and the treated group of 16 animals. It appears from Table 4 that during the 90-day treating period the average tumor volumes were considerably lower in the treated group than in the control group.

TABLE 4

Effect of D-depleted water on tumorous human HT-29 colon cells in mice

Days	Average of tumor volumes, cm ³	
	Control	Treated
1	0.16	0.16
20	0.81	0.45
35	2.28	1.88
55	5.82	4.85
70	8.09	6.80
85	19.48	10.96
90	20.74	12.35

Summarizing the results of the animal tests it can be stated that applying the pharmaceutical products according to the invention for curing tumorous diseases a recovery rate of about 50% can be attained in the early stage of the disease and the survival period can be increased by 20-30% in an advanced stage of the disease. These results can be further improved by applying water containing even less deuterium.

The products according to the invention can be applied for therapeutic purposes in forms containing the active ingredient together with inert, physiologically acceptable carriers and/or auxiliaries. The active ingredient can be transformed

into compositions for oral administration (e. g. solutions, emulsions, suspensions, etc.), parenteral administration (e. g. injectable solutions) or rectal administration (solutions for rectal infusion). The active ingredient can be applied for external use, too, e. g. in the form of ointments.

The pharmaceutical products according to the invention can be prepared by applying known methods conventionally used in the pharmaceutical industry, that is by mixing the active agent and the inert inorganic or organic carriers and then by processing the mixture into galenics.

Water or ethanol can be preferably used as liquid carrier.

The pharmaceutical products can contain also further auxiliaries conventionally used in the pharmaceutical industry, e. g. wetting agents, sweeteners, fragrances, buffering agents etc.

The medicinal solutions according to the invention can be prepared by applying known methods generally used in the food industry for preparing fruit juices, syrups, soft drinks and beer, that is by admixing the active agent with the basic materials of the soft-drink and beer industry such as fruit juices, juice concentrates, flavouring agents, sweeteners, fragrances, essential oils as well as other additives and auxiliary agents conventionally used in the soft-drink and beer industry.

The daily dosage of the pharmaceutical products according to the invention can be varied within wide ranges, depending on several factors, e. g. the activity of the active ingredient, the condition and age of the patient, the type of the tumour, the degree of malignity, etc. In the case of a patient of 70 kg body mass the oral daily dose is 1-2 litres of deuterium-depleted fluid containing deuterium in a concentration range of from 0.1 ppm to 110 ppm.

The D-depleted water can contain e. g. 30-50 g of carbohydrate and other flavouring agents or fragrances in order to make the pharmaceutical products tastier.

In case of injectable solutions the daily dose can be up to 2-6 litres and the deuterium concentration of the water can change between wide intervals (0.1-110 ppm). In general, the deuterium concentration of the water in the patient's body should be decreased by at least 0.5 ppm daily in order to assure the desired therapeutic effect. These doses are only of informative character, and the dose to be applied should be always prescribed by the medical attendant.

The main advantages of the product and process according to the invention are as follows:

- The process gives a chance for intervening directly into the regulation mechanism of cell-proliferation in a natural way.
- Tumorous diseases can be prevented and cured by using the pharmaceutical products according to the invention.
- The components of the product have no toxic side effects.
- No wastes harmful for the environment are produced in the manufacturing process.
- The process can be carried out in a technologically simple way.
- Mutant cells are not generated in the course of the therapeutic treatment since the active ingredient is not mutagenic. (Most of the cytostatics applied so far have a strong mutagenic character, therefore frequently inducing novel tumours.)
- The application of the pharmaceutical products according to the invention results in recovering and not in delaying the development of the disease.

The product and process according to the invention are illustrated in more detail in the following examples, without limiting the scope claimed.

EXAMPLE 1

Production of D-depleted water by electrolysis

An aqueous 15-20 w/v % KOH solution is electrolyzed by direct current at a potential of 2-5 V with a cathode and an anode separated from each other. The hydrogen evolving on the cathode and containing deuterium in a decreased concentration is burnt and the steam being formed is condensed and separately collected. The so-obtained water has a deuterium content of 30-40 ppm which is decreased to 6-20 ppm by a further electrolysis.

The so-obtained product can be used for assuring the fluid demand of patients suffering from tumorous diseases and—as starting material—for producing compounds of decreased deuterium content.

The end-product of the process is distilled water, therefore it is preferable to add essential salts before using it for human consumption. The end-product can be advantageously supplemented with a salt mixture containing 1000 mg of sodium, 200 mg of potassium, 160 mg of calcium, 88 mg of magnesium, 650 mg of phosphorous and 600 mg of chlorine, calculated to 1 litre.

EXAMPLE 2

Production of D-depleted water by distillation

The water is distilled in a fractionating column of 30-50 plates under a pressure of 50-60 millibar and at a temperature of 45° to 50° C. The reflux value is maintained between 12 and 13 in the course of the distillation. To keep the D concentration at a low level a tenfold dilution at the bottom is applied during the distillation. Applying these parameters, the deuterium concentration of the head-products is 20-30 ppm. The deuterium content of the water can be decreased to 1-10 ppm by further increasing the plate number and/or by repeating the distillation process.

The end-product of the process is distilled water, therefore it is suitable to add essential salts before using it for human consumption. The salt mixture of Example 1 can be advantageously applied for this purpose.

EXAMPLE 3

Production of D-depleted physiological salt solution

8.5 g of NaCl were added to 1 litre of distilled water prepared according to Example 1 or 2. The physiological salt solution is usually applied as an infusion solution after carrying out the usual sterilization process. Applying this product form the daily dose can be increased to 2-6 litres in serious cases.

EXAMPLE 4

Production of D-depleted fruit juices

Distilled water prepared according to Example 1 or 2 and containing 20-30 ppm of deuterium is mixed with water and fruit juice concentrate as follows:

- 0.8 part by volume of water containing 20-30 ppm of deuterium+0.2 part by volume of fruit juice concentrate (the final concentration of deuterium is about 45-50 ppm);
- 0.5 part by volume of water containing 20-30 ppm of deuterium+0.2 part by volume of fruit juice concentrate+0.3 part by volume of normal water (the final concentration of the deuterium is about 85-90 ppm);
- 0.3 part by volume of water containing 20-30 ppm of deuterium+0.2 part by volume of fruit juice concentrate+0.5 part by volume of normal water (the final concentration of the deuterium is about 105-110 ppm).

Starting from water containing deuterium in a lower concentration, fruit juices containing even lower deuterium concentration can be prepared.

EXAMPLE 5

Production of D-depleted soft drinks with carbonic acid content

Distilled water prepared according to the Example 1 or 2 and containing 20-30 ppm of deuterium is mixed with soft drink concentrate containing 50 g/l of sugar, 5% by volume of orange juice, 6 g/l of carbonic acid, 1 g/l of citric acid, 500 mg/l of ascorbic acid and 500 mg/l of natural flavouring agents, as follows:

- 0.8 part by volume of water containing 20-30 ppm of deuterium+0.2 part by volume of soft drink concentrate (the final concentration of the deuterium is about 45-50 ppm);
- 0.5 part by volume of water containing 20-30 ppm of deuterium+0.3 part by volume of normal water+0.2 part by volume of soft drink concentrate (the final concentration of the deuterium is about 85-90 ppm);
- 0.3 part by volume of water containing 20-30 ppm of deuterium+0.5 part by volume of normal water+0.2 part by volume of soft drink concentrate (the final concentration of the deuterium is about 105-110 ppm).

Starting from water containing deuterium in a lower concentration, soft drinks containing deuterium in a lower concentration can also be prepared.

EXAMPLE 6

Production of beer having decreased deuterium content

Barley is first soaked in water having 0.1 to 110 ppm deuterium content for producing malt, then germinated at a temperature of 5°-15° C. in a sheet having a thickness of 5-15 cm and under good aeration conditions.

The germinated barley is dehydrated at a temperature between 56° C. and 75° C., separated from the roots of germ roots, and then ground. The ground malt is mixed with a suitable amount of water having a deuterium content of 0.1-110 ppm. The mixture is heated at a temperature between 50° C. and 75° C., then filtered and brewed with hop. The hopped beer is filtered, cooled and then inoculated with prepropagated *Saccharomyces cerevisiae*. The period of the primary fermentation process at 5°-6° C. lasts 10-14 days. The secondary fermentation process is carried out in sealed drums at 0° C. for some weeks, then the so-prepared beer is filtered, bottled and pasteurized.

The deuterium content of the beer prepared according to this Example depends on the deuterium content of the applied water which influences the deuterium content of the ethanol and other components, too.

EXAMPLE 7

Production of a hydrating ointment of decreased deuterium content

The hydrating ointment is produced in a conventional way by using D-depleted water. The composition of a generally usable hydrating ointment related to 1000 g of the product is as follows:

Unguentum hydrosom	550 g
Unguentum oleum	150 g
Aqua destillata containing 30-40 ppm deuterium	300 g

I claim:

1. A pharmaceutical composition for treating a tumorous disease, comprising a pharmaceutically acceptable aqueous solution having a deuterium concentration of from 0.1 to 110 ppm as active agent.

2. The pharmaceutical composition of claim 1 wherein said pharmaceutically acceptable aqueous solution is a physiological salt solution.

3. The pharmaceutical composition of claim 1 wherein said pharmaceutically acceptable aqueous solution is selected from the group consisting of fruit juice, soft drink, and beer.

4. A process for preparing the pharmaceutical composition of claim 1, comprising the steps of:

producing by at least one of electrolysis and distillation water having a deuterium concentration less than the concentration of deuterium in natural water, and transforming said water to one of a pharmaceutical product and a medicinal solution.

5. The process of claim 4, wherein:

said pharmaceutical product is a physiological salt solution, and said pharmaceutical product has a deuterium content of 0.1 to 110 ppm.

6. The process of claim 4, wherein:

said medicinal solution is selected from the group consisting of fruit juice, soft drink and beer, and said medicinal solution has a deuterium content of 0.1 to 110 ppm.

7. The process of claim 4, further comprising the step of formulating said pharmaceutical composition into a form selected from the group consisting of an injectable solution, an infusion solution, a syrup, a juice, and a hydrating ointment.

8. A method of treating a tumorous disease in an organism comprising the step of administering to said organism an effective amount of the composition of claim 1.

9. The method of claim 8 wherein said tumorous disease has a classification selected from the group consisting of a breast cancer, a prostate cancer, a colon cancer, a liver cancer, an ovarian cancer, a sarcoma, a brain cancer, a stomach cancer, a uterine cancer, a kidney cancer, a lung cancer, a rectal cancer, a skin cancer, a myeloid leukemia, a leukocytic leukemia, a monocytic leukemia, an aleukothemic leukemia, a myeloma, a lymphoma, a bone cancer, a myeloma, a myoma, an endothelialoma, an epithelioma, a carcinoma, and a melanoma.

10. A method for treating a neoplastic disease comprising: depleting a deuterium content of water in a material to produce a treatment material having a deuterium concentration substantially below a naturally occurring deuterium level in said material; and

administering said treatment material to a patient in a sufficient quantity to reduce a deuterium concentration in said patient to a pharmaceutically effective level below said naturally occurring deuterium level, whereby said neoplastic disease is treated.

11. A method for treating neoplastic disease comprising the steps of:

manufacturing a composition having a water content deuterium concentration of less than a normal physiologic concentration; and

administering said composition to a patient, said step of administering including administering a sufficient quantity of said composition whereby a general deuterium concentration in said patient drops to a level below said normal physiologic concentration.

12. A method of treating a tumorous disease comprising the step of applying at least one product having a water content deuterium concentration less than a deuterium content of natural waters to a patient.

13. The method of claim 12 wherein said step of applying is at least one selected from the group consisting of injecting, infusing, feeding, drinking, and topically applying.

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14. The method of claim 12 wherein said deuterium concentration less than a deuterium content of natural waters is a concentration in a range of about 0.1 to about 110 ppm.

15. The method of claim 12 wherein said at least one product is one of a water and an aqueous solution.

16. The method of claim 15 wherein said aqueous solution is selected from the group consisting of a physiological salt solution, a reconstituted fruit juice, a soft drink, and a beer.

17. The method of claim 12 wherein said product is a formulation selected from the group consisting of an injectable solution, an infusion solution, a syrup, a juice, and a hydrating ointment.

18. The method of claim 12 wherein each said at least one product having a deuterium concentration less than a deuterium content of natural waters results from a production process where a deuterium depleted water is substituted for a water having a deuterium concentration equal to a deuterium content of natural waters.

19. The method of claim 15 wherein said aqueous solution results from a production process where a deuterium depleted water is substituted for a water having a deuterium concentration equal to a deuterium content of natural waters.

20. The method of claim 15 wherein said water is produced by at least one of electrolysis and distillation.

21. The method of claim 18 wherein said deuterium depleted water is produced by at least one of electrolysis and distillation.

22. The method of claim 19 wherein said deuterium depleted water is produced by at least one of electrolysis and distillation.

23. A method for decreasing a deuterium level in an organism comprising the step of applying a product having a water content deuterium concentration in an amount less than a deuterium content of natural water.

24. The method of claim 23 wherein said step of applying is selected from the group consisting of injecting, infusing, feeding, drinking, and topically applying.

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25. The method of claim 23 wherein said deuterium concentration less than a deuterium content of natural waters is a concentration in a range of about 0.1 to about 110 ppm.

26. The method of claim 23 wherein said at least one product is at least one of water or an aqueous solution.

27. The method of claim 26 wherein said aqueous solution is selected from the group consisting of a physiological salt solution, a fruit syrup, a soft drink, and a beer.

28. The method of claim 23 wherein said product is in a form selected from the group consisting of an injectable solution, an infusion solution, a syrup, a juice, and a hydrating ointment.

29. The method of claim 23 wherein said product results from a production process where a deuterium depleted water is substituted for a water having a deuterium concentration equal to a deuterium content of natural waters.

30. The method of claim 26 wherein said aqueous solution results from a production process where a deuterium depleted water is substituted for a water having a deuterium concentration equal to a deuterium content of natural waters.

31. The method of claim 26 wherein said water is produced by at least one of electrolysis and distillation.

32. The method of claim 29 wherein said deuterium depleted water is produced by at least one of electrolysis and distillation.

33. The method of claim 30 wherein said deuterium depleted water is produced by at least one of electrolysis and distillation.

34. A method of treating a tumorous disease in an organism, comprising the step of administering to said organism an effective amount of the composition of claim 1, wherein said tumorous disease has a classification selected from the group consisting of a breast tumor, a lung tumor, a rectal tumor, a colon tumor, and a prostate tumor.

WATER AND LIFE

THE UNIQUE PROPERTIES OF H₂O

edited by
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behavior of a protein containing 400 amino acids (hence "slightly discrete"). Thus, one can search a fitness landscape in small steps with a biopolymer. Similar mechanisms are accessible to change the structures of metabolites, less discretely, but still within a largely stable environment.

No such slightly discrete stages are possible to evolve water. Other molecules exist, of course, that might replace water. The simplest arise from combinations of second row elements with hydrogen (the "ices," as they are known to planetary scientists, including BH₃, CH₄, NH₃, OH₂, and FH).

These ices do not, however, provide a set of molecules that are slightly different in their physical properties. These mutant forms of water are extremely different from water in their properties, even by naive metrics. These different properties include melting points, boiling points, and intrinsic reactivities. Because the ices cannot change, even slightly, their structure and properties, the function-structure landscape that includes them as points must be rugged. A rugged structure-function landscape defeats the Darwinian search for optimality. This implies that ices could not have evolved in a Darwinian sense to serve themselves, or their biological contents.

10.8 IS WATER WELL SUITED FOR LIFE?

This discussion, of course, assumes immutable physical law. This assumption can be relaxed, much as it is being relaxed in physics. If we assume that the properties could have been different, we can ask whether a different *kind* of water might be better suited to life.

In 1986, Barrow and Tipler (1986) addressed this question as part of their larger discussion about whether physical laws in general are well suited to support life. These might include physical laws that determine, *inter alia*, the properties of water. Thus, the universe, and more specifically the chemistry in the universe, might be adapted to facilitate life.

Barrow and Tipler (1986) discuss two general ways to view a match between the physical properties of water and the requirements for a solvent to support life. A match of some sort is clearly necessary for a sentient being to exist, and therefore be able to perceive that the match exists. Humans would not be alive to comment on the suitability of water as a solvent for life if it were not suited (in some sense) for life.

This need not be cast as a Darwinian model, but it might be. To be Darwinian, the physical laws that we know might have been chosen from a variety of law possibilities from a variety of universes. The variant universes must have progeny that have slightly different physical laws, the differences must themselves be heritable, and the fitter variant universes must survive to the exclusion of less fit universes. Under this particular version of the anthropic hypothesis, a large number of alternative universes exist, some whose ices behave in ways conducive for the emergence of life, and others whose ices do not.

The second view falls within the category of intelligent design. This version of the hypothesis requires a chooser, a supernatural entity (using this adjective in the literal sense) that arranges to have a universe in which life might emerge, and therefore chooses the physical laws (including properly behaved ices) that would permit life to emerge.

Synthesis would be a useful paradigm here as well. If we could synthesize alternative universes with different physical laws, we might run experiments to learn whether the alternatives are indeed more or less suited for life, just as we experimented with alternative synthetic DNA. Unless reality is very different from how we perceive it, this is not possible. To test these hypotheses, we do not have access to synthesis to manage our prejudices and to drive discovery. We can, however, ask simpler questions. For example, if water had different properties, how would the molecular structures of life need to be adjusted?

10.9 THE PROPERTIES OF WATER ARE NOT VERY FINELY TUNED TO LIFE

Discussions in physics have suggested that physical laws are very finely tuned to support life. In some cases, if physical parameters were different by a few percent, the cosmos would be very different from the way it is now.

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We know that this is not true for water as a biosolvent. We can change (and scientists have changed) slightly the properties of water by replacing the protium isotope of hydrogen with deuterium, creating heavy water (a synthetic water, in a sense). Deuterated molecules have different properties: in some cases they react seven times slower than protiated analogs. Thus, the perturbation arising through the replacement of a protium by a deuterium is not negligible.

We can (and scientists have) put living systems adapted to protiated water into deuterated water to see what happens (Katz et al., 1962, 1964). Living systems often do not do too well at first. But if the life is microbial and has access to adaptive responses (epigenetic adaptation seems to be sufficient), the system changes to perform as well as it did in protiated water. There is little doubt that life, even at the level of the mouse, would become fully adapted to the tweaked water, as well as it is to undeuterated water, if given full access to Darwinian processes.

The many studies by Katz and his coworkers of life in deuterated water were so detailed that the issue has not been revisited in recent years. We might (in principle) repeat the experiment with carrier-free tritiated water, another synthetic water, as a solvent. Replacing protium by tritium creates a larger perturbation, with tritiated molecules reacting typically 10 to 20 times slower than their protiated analogs. The experiment has not been done. But there is little doubt that concerning the chemical properties of the modified solvent, life could adapt perfectly well.

At this point, the experiments must stop. No more slightly discrete variations are available in water to do any more analyses of this type. The experiments with deuterated water make indisputable, however, the view that the properties of water are not as finely-tuned as has been proposed for some fundamental physical parameters, such as the fine structure constant (Barrow and Tipler, 1986).

10.10 MORE INTELLECTUAL PROCESS

The inability to synthesize alternative solvents with modestly changed properties leaves those asking about the intrinsic suitability of water for life with only a few intellectual tools; analysis and modeling are among them. Pure analysis (in ancient Greece, "metaphysics") relies heavily on concepts of "elegance" and "intuition." These are themselves largely reflections of the perspective of the analyzer.

The limitations of pure analysis are well known in physics. For example, Ginsparg and Glashow pointed out two decades ago that the intimate interconnection of space and time would not likely have been discovered had Maxwell's equations not emerged from experiment (Ginsparg and Glashow, 1986). We certainly would not have generated the idea of the quantum without the preceding century of chemistry, which relied on synthesis and experiment, to identify the elements from molecules to enable spectroscopy. Experiment is needed to drive ideas away from prejudice and toward novelty.

Two analytical approaches are clearly unable to address this question. First, the observation that all of the life that we know uses water is not a reliable guide. As noted above, life on Earth has almost certainly evolved in water. In doing so, it undoubtedly exploited opportunities to change its genetically encoded molecules and metabolites, slightly discretely, to become optimal for life in water. Thus, it is not surprising, given the Darwinian paradigm, to find that terran life is well adapted to water, at least to the extent to which various chemical constraints permit it.

Furthermore, terran life is the only life that we know. Therefore, given the anthropocentric tendencies of human thought processes, our models for life are influenced (to the point of being determined) by our experience with terran life. Indeed, given the general lack of creativity of the human mind, it is hard to find a model for life that does not resemble the terran life that we know; the aliens on *Star Trek* generally resemble Hollywood actors with prostheses. It is natural to slip from the Darwinian axiom (that life has adapted to water) to the assumption that water must be adapted to life.

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ISOTOPE HYDROLOGY

A Study of the Water Cycle

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Chapter 2

The Isotopes of Hydrogen and Oxygen

Hydrogen and oxygen have a number of isotopes, both stable and radioactive. The major isotope of hydrogen with a mass of 1, (^1H), occurs in the hydrosphere at a mass abundance of 99.985%. It is accompanied by about 0.015% of the heavy isotope ^2H whose particular name is Deuterium, designated D in the older literature. An even heavier isotope of mass 3 (^3H), named Tritium, is unstable to β decay with a half-life of 12.43 years; this is the value usually accepted even though more recent measurements suggest the value of $T_{1/2} = 12.23$ years (Lucas and Unterwieser, 2000). As this half-life is compatible with the holdup time in many subsurface reservoirs, Tritium is also widely used in hydrologic studies as a tracer.

The most abundant oxygen isotope, ^{16}O , whose mean mass abundance in the hydrosphere is given as 99.762%, is accompanied by a number of stable and radioactive isotopes. The radioactive oxygen isotopes ^{14}O , ^{15}O , ^{19}O and ^{20}O all have half-lives of only seconds and are thus too shortlived to be of any significance in the study of the hydrologic cycle. However, the two stable heavy isotopes of oxygen, ^{17}O and ^{18}O , whose average abundances are 0.0379% and 0.200%, respectively, are the powerhouses of isotope hydrology.

Given the five stable isotopes of hydrogen and oxygen, one will have nine isotopic water molecules (termed isotopologues in the recent literature) ranging from a mass of 18 for $^1\text{H}_2^{16}\text{O}$ to mass 22 for $^2\text{H}_2^{18}\text{O}$. Assuming statistical distribution of the isotopic species in water, the abundances shown in Table 2.1 are proposed. It is clear that one is concerned mainly with the molecules $^1\text{H}^2\text{H}^{16}\text{O}$, $^1\text{H}_2^{17}\text{O}$, $^1\text{H}_2^{18}\text{O}$ and, of course, $^1\text{H}_2^{16}\text{O}$. The doubly labeled molecules $^1\text{H}^2\text{H}^{17}\text{O}$, $^1\text{H}^2\text{H}^{18}\text{O}$ and $^2\text{H}_2^{16}\text{O}$ and even more so the triply substituted molecules $^2\text{H}_2^{18}\text{O}$ and $^2\text{H}_2^{17}\text{O}$ can be disregarded at the level of natural abundance of these isotopes.

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Table 2.1. Isotopic water species and their relative abundance.*

Mass	Molecule	Rel. Abundance
18	$^1\text{H}_2^{16}\text{O}$	0.99731
19	$^1\text{H}^2\text{H}^{16}\text{O}$	3.146×10^{-4}
19	$^1\text{H}_2^{17}\text{O}$	3.789×10^{-4}
20	$^1\text{H}^2\text{H}^{17}\text{O}$	1.122×10^{-7}
20	$^2\text{H}_2^{16}\text{O}$	2.245×10^{-8}
20	$^1\text{H}_2^{18}\text{O}$	2.000×10^{-3}
21	$^2\text{H}_2^{17}\text{O}$	—negligible
21	$^1\text{H}^2\text{H}^{18}\text{O}$	6.116×10^{-7}
22	$^2\text{H}_2^{18}\text{O}$	—negligible

(* Assuming equilibration during disproportionation reactions)

{Note that the abundance of molecules which are singly substituted by Deuterium, e.g. $^1\text{H}^2\text{HO}$, is twice that of the product of the atomic abundances}. As given by Coplen *et al.* (2002).

Because of the measurement procedure by mass spectrometry, one often uses isotope (abundance) ratios, R , rather than the conventional concentration units, C . $R = N_i/N_j$ where N_i and N_j are the numbers of the rare and common isotope species, respectively. For the case of low natural abundances of the heavy isotope (so that the number of double-labelled molecules such as $^2\text{H}_2\text{O}$ is low) this practically equals the atom-% of the two isotopes.

The concentration of the isotopic molecules, C , is defined as:

$$C = (\text{number of isotopic molecules}) / (\text{total number of molecules}),$$

and this is related to the isotope ratio as follows: $C = R/(1 + R)$.

One uses a superscript before the symbols to refer to the isotope under consideration while the molecule involved follows in paranthesis. Thus, for example, $^{18}\text{R}(\text{H}_2\text{O})_v$ stands for the ratio of $[\text{H}_2^{18}\text{O}]/[\text{H}_2^{16}\text{O}]$ in water vapour, while $C(\text{H}_2^{18}\text{O})_v$, the concentration of H_2^{18}O , is given by the ratio of $[\text{H}_2^{18}\text{O}]/\Sigma$ [all isotopic water molecules] which can also be written as $C(\text{H}_2^{18}\text{O}) = [\text{H}_2^{18}\text{O}]/[\text{H}_2\text{O}]$, recalling that H or O without any mass assignment signifies the total amount of that element. In the case of natural waters where the abundances of the "heavy" isotopes are small, these two scales do not differ in a significant way.

Even though these isotopes are stable and not subject to radioactive decay, they can be products or reactants in nuclear reactions initiated by natural radioactivity or by cosmic radiations. Moreover, hydrogen is accreted from the solar wind with isotopic abundances quite different from the terrestrial ones. However, these interactions are believed to be of only

Table 2.2. Properties of isotopic water molecules.

	$^*\text{H}_2^{18}\text{O}$	$^2\text{H}_2^*\text{O}$	$^1\text{H}^2\text{H}^*\text{O}$
Density $[\text{g.ml}^{-1}]$ at 30°C ^b	1.107845	1.10323	1.04945 ^a
Temperature at maximum density $[\text{C}^\circ]$ ^b	4.305	11.24	
Boiling point $[\text{C}^\circ]$ ^c	100.14	101.42	
Melting point $[\text{C}^\circ]$ ^b	0.28	3.81	
Self-diffusion coefficient through liquid water $[\text{D} \cdot 10^3 (\text{cm}^2 \cdot \text{s}^{-1})]$ at 25°C ^d	2.66	2.34	
Molecular diffusivity through air relative to the diffusivity of $^*\text{H}_2^*\text{O}$ ^e	0.9723		0.9755

Legend:

* — element at natural abundance

a — estimate by interpolation

b — (Steckel and Szapiro, 1963)

c — (Szapiro and Steckel, 1967; Zieborak, 1966)

d — (Wang *et al.*, 1953)

e — (Merlivat, 1978)

minor consequence for the average terrestrial abundance, which can be considered invariant to a first approximation.

The observed variability in the isotopic abundance within the hydrologic cycle, and in other materials in the lithosphere or biosphere, which range about $\pm 50\%$ around the mean ^{18}O abundance and from $+300\%$ to -500% in the case of Deuterium (^2H), are the result of isotope fractionation processes within the system, to be discussed in the following chapters. Throughout the hydrological cycle, the phase transitions of water between ice, liquid and vapour are the most important processes to be reckoned with.

Some properties of the isotopic molecules of water are shown in Table 2.2.

The ratio of densities of $\text{H}_2^{18}\text{O}/\text{H}_2\text{O}^*$ and $^2\text{H}_2\text{O}/\text{H}_2\text{O}^*$ is close to $d_{30}^{30} = 1.12653$ and 1.108018 , respectively. These values are close to the mass ratios of 1.1110 and 1.1117 . The good agreement is the result of the fact that inter-atomic distances are almost invariant under isotopic substitution. The molecules $^1\text{H}^2\text{H}^{16}\text{O}$ and H_2^{17}O have properties in between those of the heavy and light water molecules.

2.1. Measurement techniques

Mass is the most immediately discernible difference between the stable isotopic species. Thus it is only natural that in the beginning (following the

Box 2.3. (Continued)

of the isotopes, at a reproducibility of 2.10^4 (Hagemann *et al.*, 1970). For many other "reference materials" and other isotopic species there are no absolute isotope ratio determinations and their comparison to SMOW and other standards depends on estimates of the isotope fractionation between the different co-existing materials. However, recently, the isotope abundance measurements have been much improved and reproducibilities of 10^{-4} to 10^{-5} can be obtained for the measurement of absolute ratios of oxygen isotopes in O_2 gas. Since fluorination techniques can produce O_2 gas from water, carbonates, sulfates, silicates and phosphates at close to 100% yield, thus being free of fractionation effects, it has been suggested that atmospheric O_2 gas from areas of either sources or sinks of oxygen be used as a primary standard for the oxygen isotopes. Following the accurate calibration of such a standard by means of synthetic mixtures of the isotopes concerned, a comparison can then be made between CO_2 produced by conversion of the standard to CO_2 with the CO_2 produced in each case by the accepted analytical procedures, i.e. H_2O-CO_2 equilibration, acid decomposition of carbonates, etc (Gat and DeBievre, 2002).

2.2. The natural abundance of isotopes of oxygen and hydrogen

The two Boato diagrams (Figs. 2.1 and 2.2) show the range of the abundance of the heavy isotopes in natural materials (Boato, 1961). $\delta^{18}O$ values span a range of $\pm 50\%$ around SMOW, with most of this range pertaining to the waters of the hydrologic cycle. The range of δ^2H values is considerably larger. In the water cycle, the meteoric waters, i.e. the precipitation and water derived directly from precipitation are relatively depleted in the heavy isotopes, whereas surface water exposed to evaporation and water in geothermal systems fall on the enriched side of the scale.

The average of the present hydrosphere is estimated at $\delta(^{18}O) = -0.64\%$. The value of the primordial water, i.e. that water first condensed over the primitive earth and still possibly present deep down under the earth's surface, has been of great interest. The value suggested is $\delta^{18}O = +5\%$ and $\delta^2H = -55\%$ and is based on the isotopic composition of old rocks formed at high temperatures (so that no isotope fractionation can be expected) and on the value of water in magmatic exhalation. The evolution in the δ value of the hydrogen isotopes from the primordial to the present day hydrosphere is explained by the slow but preferential loss to space of 1H atoms from the exosphere. In the case of the Oxygen-18, the loss of heavy oxygen can be accounted for by the deposition of carbonates

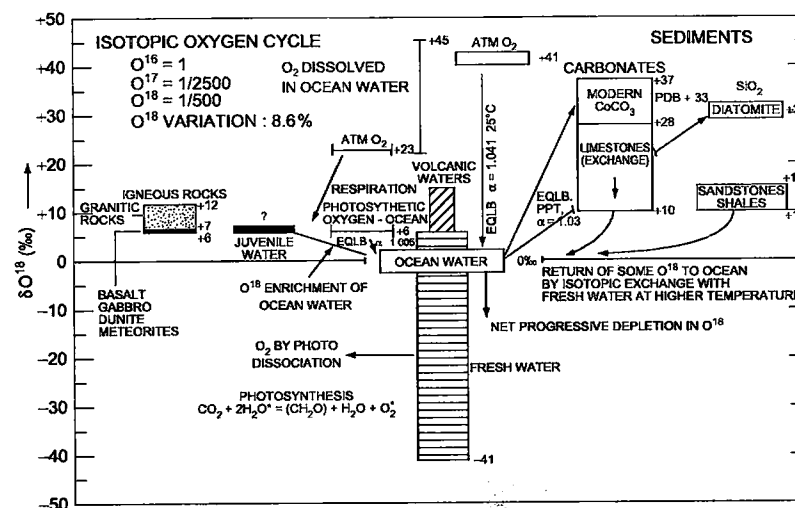


Fig. 2.1.

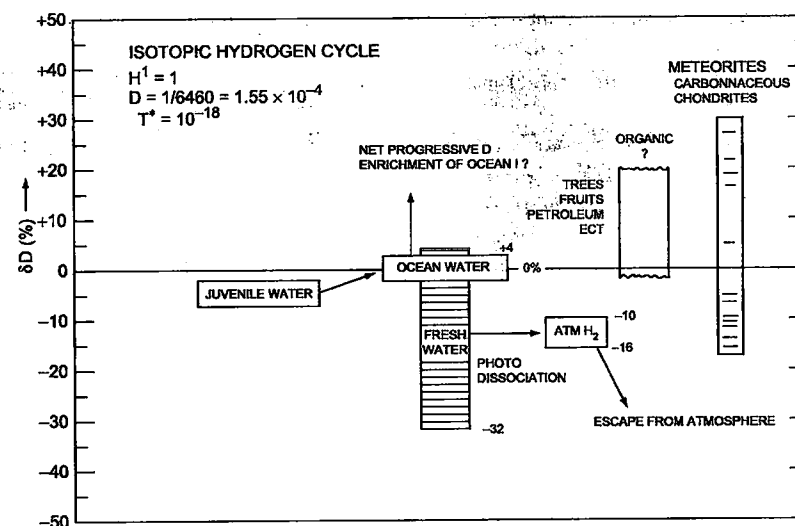


Fig. 2.2.

Box 2.4. Oxygen-17 Labelled Water.

Classical equilibrium isotope fractionation theory (Bigeleisen, 1962) predicts that the isotopic changes of the Oxygen-17 labelled molecules would be about one half that of the equivalent ones labelled by Oxygen-18. The prevalent measurement techniques for Oxygen-17, namely by NMR or Mass Spectrometry on CO_2 (where the molecule $^{13}\text{C}^{16}\text{O}_2$ of mass 45 masks the changes in abundance of the sought after molecule $^{12}\text{C}^{16}\text{O}^{17}\text{O}$), were not sensitive enough to detect any deviations from such a relationship within the hydrologic cycle. However, it was shown that extra-terrestrial abundances of ^{17}O in meteorites differed markedly from the expected ones (Clayton, 1993). Moreover, measurements of the isotopic composition of stratospheric oxygen and CO_2 (cf. Thiéman et al., 1995) established an anomalously large enrichment of the ^{17}O -labelled molecules of oxygen and ozone; this was shown to be the result of a mass-independent fractionation by photochemical reactions in the upper atmosphere.

In order to exploit this isotopic tracer pulse for studies of the mixing and incorporation of the high altitude oxygen in the terrestrial geochemical cycles (Luz et al., 1999), the Oxygen-17 analytic procedures had to be improved. This was achieved by using Oxygen gas for the measurement in the MS, which was produced from the water by electrolysis (Meijer and Li, 1998) or by a fluorination technique, with CoF_3 as the fluorination agent. Barkan and Luz (2005) perfected this procedure to obtain a precision of 0.01‰ to 0.03‰ for both $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$. Using this method, they determined the fractionation factor $^{17}\alpha$ and $^{18}\alpha$ for the liquid/vapour equilibrium and found the ratio of $\ln^{17}\alpha/\ln^{18}\alpha$ to be constant with a value of 0.529 ± 0.001 over the temperature range of 11.4° to 41.5°C. Moreover, a large suite of samples of meteoric waters followed a similar relationship. However, the relationship between the fractionation effects of the diffusion of the isotopic water molecules through air during the evaporation of water followed a smaller slope of 0.518, so that these two effects can be distinguished by measuring both the ^{17}O and ^{18}O isotopes. Examples of use of this methodology is found in Landais et al. (2008).

and other sedimentary minerals (which are relatively enriched in the heavy isotope) from the world ocean over the geologic time span.

The ^{17}O abundance is estimated at about 37.4×10^{-3} . By and large, the variability in ^{17}O mimics that of ^{18}O at about one half the amount of change, since most of the isotope fractionation occurs in a mass-dependent fashion. However, there is mounting evidence that some mass-independent fractionation takes place during photochemical interactions in the higher atmosphere. The case of the ^{17}O labeled waters is discussed in Box 2.4.

Chapter 3

Isotope Fractionation

Introduction

Due to the difference in the properties of the isotopic atoms and molecules, be it rates of motion, frequencies of inter-molecular vibrations, rotations or stability of chemical bonds, the relative abundance of the isotopes in the source material and the product of a dynamic process is different. Even in the so-called *equilibrium systems*, where the amount and concentration of the chemical compounds involved do not change, one encounters differences in the abundance of the isotopic species in the various components of such a system. The term "*Isotope Fractionation*" is used to denote any situation where changes in isotopic abundances result.

Based on the process which is responsible for the change, one distinguishes between *equilibrium* fractionation (also termed thermodynamic fractionation), and *kinetic* and *transport* fractionations. These will be discussed in the following sections. All of these are *mass-dependent* effects, in the sense that there is a quantitative difference in the rates or extent of the isotope fractionation which depends directly on the mass difference between the atoms or molecules involved. Where a number of isotopes of the same element are involved, such as the case of ^{18}O , ^{17}O , ^{16}O or ^{14}C , ^{13}C , ^{12}C , the mass dependency is reflected in the degree of enrichment or depletion of the isotope with intermediate mass, which is in between that of the heavier and lighter isotopes (Bigeleisen, 1962).

The mass dependency also explains the fact that some of the fractionation effects involving the hydrogen isotopes are larger by almost one order of magnitude when compared to those involving the oxygen or carbon isotopes because of the relatively large ratio in mass of 2:1 in the case of the

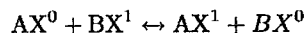
hydrogen isotopes compared to 18:16 and 13:12, respectively, for the oxygen and carbon isotopes.

There are, however, two situations where *mass-independent* fractionation is encountered. The first situation is when a nuclear interaction occurs with one or the other of the isotopes being either the reactant or product. The other case is that of a photo-chemical reaction, whereby the bond involving just one of the isotopic species is preferentially excited by radiation of the appropriate wavelength.

Changes in the (averaged) isotope composition of different compartments or fluxes in the hydrological cycles can also be encountered without any fractionation when these compartments are not well mixed or equal in either time or space, and then partially utilized. Such a process, termed a *selection process* to distinguish it from a fractionating one, is one based for example on the seasonal changes in the isotope composition of atmospheric, surface or soil waters, as described in Chapter 8.

3.1. Isotope fractionation under equilibrium conditions (Thermodynamic fractionation processes)

Compounds A and B with a common element (with more than one isotopic species) which are in equilibrium with respect to the exchange of the isotopes between them, may have different isotopic compositions, $R(A)$ and $R(B)$ resulting from differences in bond strength of the isotopic species. An isotopic fractionation factor is then defined as follows: $\alpha_{(A-B)} = R(A)/R(B)$. In analogy to the reversible chemical reactions at equilibrium (exchange reactions), one can define a thermodynamic constant of the isotopic exchange reaction:



where X^0 and X^1 stand for the two isotopes of the common element between the molecules concerned, as follows:

$$K_x = \frac{[AX^1] \cdot [BX^0]}{[AX^0] \cdot [BX^1]} \quad (3.1)$$

The brackets [] signify the thermodynamic activity of the component concerned.

K_x equals $R(A)/R(B)$ to the extent that one can assume no isotope effect on the activity coefficients ratio for the two chemical species; this, however, is a first approximation at best.

The same principle can be applied also to the equilibrium between two phases of some material, such as water in its three phases: vapour, liquid and solid (ice). The special case of the relationship between the concentration and activity fractionation factors in the case of saline solutions was discussed in Box 2.2.

These equilibrium fractionation effects are dominated by the zero-point energies of the binding energies; being quantum mechanical effects they are appreciable at lower temperatures and practically disappear at higher temperatures, typically following a rule such as:

$$\log_e \alpha = C_1/T^2 + C_2/T + C_3 \quad (3.2)$$

where T is the temperature expressed as ° Kelvin.

For the case of the vapour to liquid equilibrium of water, the following relationships were determined by Majoube (1971):

$$\log_e {}^{18}\alpha_{L/V}: C_1 = 1.137 \cdot 10^3; C_2 = -0.4156; C_3 = -2.0667 \cdot 10^{-3} \quad (3.3)$$

$$\log_e {}^2\alpha_{L/V}: C_1 = 24.844 \cdot 10^3; C_2 = -76.248; C_3 = +52.612 \cdot 10^{-3} \quad (3.4)$$

These data are shown in graphical form in Figure 3.1. The equilibrium isotope fractionation factors are often denoted as α^* or α^+ , where by a convention introduced by Craig and Gordon (1965), one uses the notation of α^* when $\alpha^* < 1$ and α^+ for $\alpha^+ > 1$.

Table 3.1 summarizes some of the fractionation factors which operate during phase transitions of water. It is evident from the magnitude of the fractionation factors during the phase transitions of water, that the resultant changes in isotope composition of hydrogen and oxygen respectively, differ by almost a factor of 10. In order to portray these then in δ (‰)-space (i.e. the plot of ${}^2\delta$ versus ${}^{18}\delta$), one foreshortens the ${}^2\delta$ axis by this factor of 10 relative to the ${}^{18}\delta$ axis. The trend lines relating the isotopic composition of vapour to the liquid or solid at thermodynamic equilibrium are seen to be temperature-dependent, ranging in slope, S (where $S = \Delta^2\delta / \Delta^{18}\delta$), from $S = 8.65$ at 0°C to $S = 7.75$ at 20°C for the liquid/vapour, and $S = 7.7$ for the vapour/ice equilibrium at 0°C . Notably, the equivalent value for the solid/liquid transition is much less than these, being $S = 5.86$.

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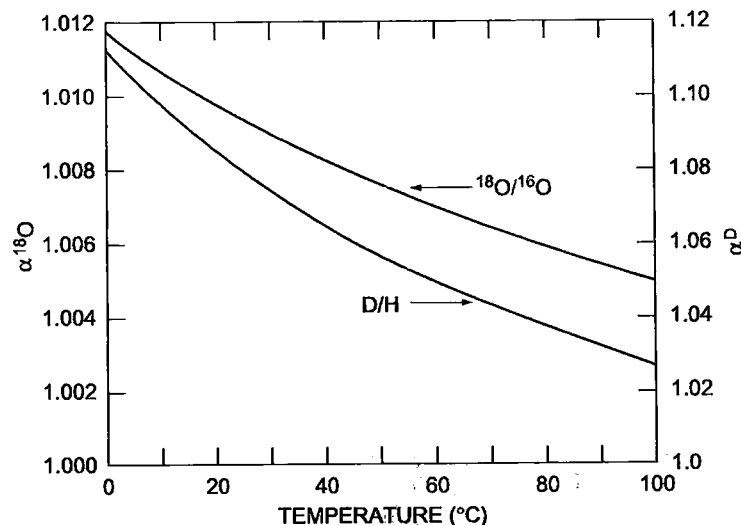


Fig. 3.1. Isotopic Species of water.

Table 3.1. Equilibrium isotope fractionation factors for phase transitions of water.

	$^{18}\alpha^+$	$^2\alpha^+$	Reference
Solid (ice)-vapour			
at 0°C	1.0152	1.132	[1]
at -10°C	1.0166	1.151	[1]
Liquid-vapour			
at +20°C	1.0098	1.084	[2]
at 0°C	1.0117	1.111	[2]
Solid (ice)-liquid			
at 0°C	1.0035	1.0208	[1]/[3]
Hydration water-solution			
Gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$)			
at 20°C	1.004	0.985*	[4]/[5]

[1] Majoube, 1971a; [2] Majoube, 1971b; [3] Arnason, 1969; [4] Fontes and Gonfiantini, 1967; [5] Sofer, 1975.

*Value refers to $^2\alpha = R(\text{water in gypsum})/R(\text{mother solution})$.

3.2. Isotope fractionation accompanying transport processes

The effect of the mass difference of isotopic molecules is expressed most directly in their transport properties. In the hydrologic cycle, differences in the rate of diffusion of the water molecules through air is of major concern. Based on the gas-kinetic theory (Chapman and Couling, 1951), where the diffusivity of molecule A through gas B is expressed by

$$D_{A,B} = \frac{\{C \cdot (1/M_A + 1/M_B) \cdot T^{3/2}\}}{\{P \cdot \sigma^2 \cdot \Omega_{A,B}\}}$$

one would expect the following ratios of diffusivities through air for the deuterated and Oxygen-18 substituted water molecules $^1\text{H}_2\text{HO}$ and H_2^{18}O , respectively, relative to the common water molecule $^1\text{H}_2^{16}\text{O}$, (under neglect of the interactive term $\Omega_{A,B}$ and on the assumption of negligible change of the molecular volume with isotopic substitution):

$$D(^1\text{H}_2^{16}\text{O})/D(^1\text{H}_2\text{H}^{16}\text{O}) = 1.015 \quad \text{and} \quad D(^1\text{H}_2^{16}\text{O})/D(^1\text{H}_2^{18}\text{O}) = 1.030.$$

Contrary to this expectation, the experimentally measured values were found quite different (Merlivat, 1978) especially for the case of the deuterated molecules:

$$D(^1\text{H}_2^{16}\text{O})/D(^1\text{H}_2\text{H}^{16}\text{O}) = 1.0250 \quad \text{and} \quad D(^1\text{H}_2^{16}\text{O})/D(^1\text{H}_2^{18}\text{O}) = 1.0306.$$

This is attributed to interactions between water molecules in the gas phase. Such an effect is even more accentuated in the liquid phase. Indeed, Wang *et al.* (1953) measured appreciable differences between the diffusion coefficients of isotopic water molecules of equal mass in liquid water, for example, the molecules $^1\text{H}_3\text{HO}$ and H_2^{18}O . Other transport properties in the liquid, such as viscosity, are similarly affected by the intra-molecular forces and especially by hydrogen-bonding.

Lately, this apparent discrepancy in the Diffusion Constant of the deuterated water molecules in air has been questioned by Cappa *et al.* (2003), based on a re-evaluation of the isotope fractionation process during the evaporation of water, as will be discussed in more detail in Chapter 4.3. However, this subject is still not agreed upon and at this stage, the evidence seems to support the more classical approach based on Merlivat (1978).

Unlike the thermodynamic and kinetic isotope effects with their strong (negative) temperature dependence, the effect of temperature on the diffusion coefficients in air is rather weak, i.e. D is proportional to $\sqrt{T}$ and the ratio of diffusivities is practically invariant with respect to temperature.

In the early literature, the fractionation effects that result from differences in the movement of the isotopic molecules were termed "*kinetic isotope fractionation*" effects, a term which nowadays is reserved for the effects resulting from the breaking and making of chemical bonds in chemical and bio-chemical reactions. The presently discussed effects are termed "*transport isotope fractionation*".

Chapter 4

Models of Isotopic Change in the Water Cycle

4.1. Closed equilibrium system

In an assemblage of N water molecules consisting of iN and jN isotopologues where j refers to the more abundant isotope species, so that $R = {}^iN/{}^jN$ and $N = ({}^iN + {}^jN)$, the distribution of the isotopologues between two coexisting phases of the water (for example between liquid and vapour or liquid water and ice) under equilibrium conditions is governed by the appropriate "Equilibrium Fractionation Factor". In the case that the two phases contain $N_{(1)}$ and $N_{(2)}$ molecules, respectively, then using a box-model formulation the distribution of the isotopes between the 2 phases, namely, the relation of $R_{(1)} = \{{}^iN_{(1)}/{}^jN_{(1)}\}$ to $R_{(2)} = \{{}^iN_{(2)}/{}^jN_{(2)}\}$, will be given at equilibrium by $\alpha = R_{(2)}/R_{(1)}$.

α is the relevant "Equilibrium Fractionation Factor" at the temperature concerned. For the case of the natural isotope abundances of either the Oxygen-18 or Deuterium, where ${}^jN \gg {}^iN$, one can write, to a good approximation, that $N = ({}^iN + {}^jN) \approx {}^jN$ and $d^iN \approx dN$.

Suppose that initially all the water is in one phase (N_0 and iN_0 molecules, respectively) and that subsequently more and more of it changes its phase under equilibrium conditions. Such is the situation when fog develops as an air mass cools as well as when clouds form in an ascending air-mass. If $0 < f < 1$ is the fraction of water remaining in Box #1, i.e. $f = N_{f,(1)}/N$, then the evolution of the isotopic composition, described by the ratio R_f , can be described by the following equation provided the equilibrium situation is maintained between the two phases throughout; note that if the temperature changes throughout the process, the controlling fractionation factor changes accordingly.

$$R_f \cdot \alpha = \{{}^iN_0 - {}^iN_f\}/\{N_0 - N_f\} = \{R_0 - f \cdot R_f\}/(1 - f) \quad (4.1)$$

In δ (‰) nomenclature where $\varepsilon = (\alpha - 1)10^3$, the equivalent formulation is:

$$\delta_f \approx \delta_0 - \varepsilon \cdot (1 - f) \quad (4.2)$$

4.2. Open equilibrium systems — the Rayleigh Equation

When material is removed from a mixed assemblage of isotopic molecules in the form of another phase under momentary equilibrium conditions, such as when water droplets are removed from moist air or the evaporate flux from a liquid, then given the initial isotopic ratio in the mixed reservoir (box) of $R = {}^iN/{}^jN$, the isotopic ratio in the effluent flux, $R_{ef} = d^iN/d^jN$, is given by the relationship:

$$\alpha = R_{ef}/R = \{d^iN/d^jN\}/\{{}^iN/{}^jN\} \quad (4.3)$$

when α signifies the Equilibrium Fractionation Factor.

When more and more of the material is removed from the reservoir, the changing isotope composition is described by the "Rayleigh Equations." This formulation is based on one given by Lord Rayleigh (1902), who 100 years ago thus described the enrichment of alcohol by distillation from an alcohol/water mixture. Even though these equations were formulated to describe a momentary *equilibrium* situation, they can be applied also in the case that fractionation factors other than the "Equilibrium Fractionation Factor" operate.

One needs to distinguish between the following situations:

- (1) Systems closed to inflow, open to outflow:
 - A) where material is removed only according to Eq. 4.3
 - B) where part of the outgoing flux occurs without any change in the isotope composition, e.g. in a bottom-leaking evaporation pond.
- (2) Systems open to both inflow and outflow:
 - C) balanced throughflow systems, i.e. influx equals efflux:
 - C1) where material is removed only according to Eq. 4.3
 - C2) where part of the outgoing flux occurs without isotope fractionation
 - D) unbalanced systems

4.2.1. Systems without inflow

The changing isotope ratio as material is removed under equilibrium conditions, based on the relationship of Eq. 4.3 and the approximation discussed above, is then given by:

$$dR/dN = d({}^iN/N)/dN = \{d^iN/dN - {}^iN/N\}/N = \{R/N\} \cdot (\alpha - 1) \quad (4.4)$$

from which it follows that:

$$d \log R / d \log N = (\alpha - 1) \quad (4.4a)$$

Defining $f = N_f/N_0$ as the fraction of material remaining in the system after part of it was removed (where N_0 is the number of molecules at the beginning of the process), then for a constant value of the fractionation factor, the evolution of the isotope ratio will be given by integration of Eq. 4.4 as follows:

$$R = R_0 \cdot f^{(\alpha-1)} \quad (4.5)$$

In δ (‰) nomenclature, since $R/R_{\text{standard}} = \{1 + \delta/10^3\}$ we have

$$d \log R = d \log \{1 + \delta/10^3\} = \{d\delta/10^3\} / \{1 + \delta/10^3\}$$

so that

$$d(\delta/10^3)/d \log f = (\alpha - 1) \cdot \{1 + \delta/10^3\}$$

For small δ values this can be approximated by:

$$\delta \approx \delta_0 - (\alpha - 1) \cdot \log f \quad (4.6)$$

The change in isotope composition can thus be used as a measure of the degree of rainout or evaporation, as the case may be.

When only a part of the efflux occurs under fractionating conditions, the change of the isotope composition according to Eq. 4.6 will not be commensurate with the actual water balance. Comparison of the expected and actual isotopic change will reveal the partitioning of outflow by the fractionating and non-fractionating modes.

4.2.2. Systems with both in- and outflow

The mass balance of such a system with both in- and outflow is given by:

$$dN = dN_{\text{in}} - dN_{\text{out}}$$

When $dN_{in} = dN_{out}$ the system is at steady state relative to the amount of material. The isotope balance is then given by:

$$d(RN)_s = R_{in} \cdot dN_{in} - \alpha \cdot R_s \cdot dN_{out}$$

Such a system can reach an isotopic steady state when $R_{in} = \alpha \cdot R_s$.

Details of such systems will be further discussed in the context of the discussion on lakes.

Figure 4.1 shows the evolution of the isotopic composition for these different model systems. Evidently, the change in isotopic composition is quite different, even though all are governed by the same fractionation factor.

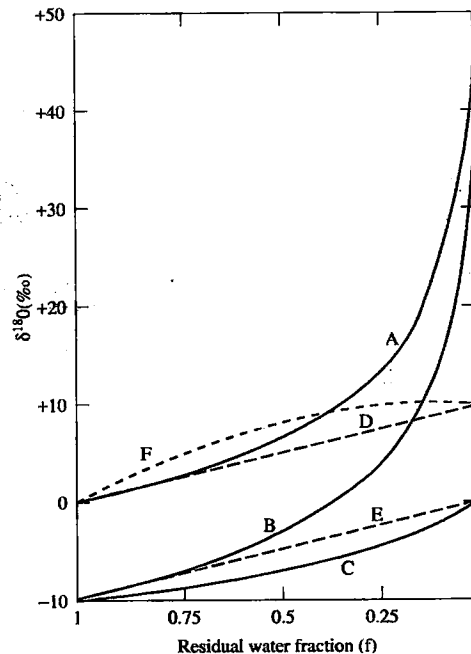


Fig. 4.1. Change of the isotopic composition of water from which vapour is removed under conditions of equilibrium-fractionation for different scenarios: Curves A,B,C: under a Rayleigh regime, showing the residual water, the instantaneous vapor flux and the total removed vapour, respectively. Curves D,E: The liquid and vapour composition, respectively, for a closed system. Curve F: A liquid approaching steady state (horizontal axis in this case represents a time axis).

4.3. Evaporation under natural conditions

The early surveys of the isotope composition of terrestrial waters reported an enrichment of the heavy isotopes in some lakes and evaporative basins. This was first attributed to a *Rayleigh process*, based on the *Equilibrium Isotope fractionation* between liquid water and its vapour. However, the high enrichments in extreme evaporative systems which were thus expected were not realized. This led to a series of experimental evaporation studies in the field and laboratory, among others by Dansgaard (1954), Friedmann *et al.* (1956), Craig Gordon & Horibe (1963), and Lloyd (1966). It was thus realized that the isotopic buildup during the process of evaporation into the atmosphere is not a simple one-way "*Rayleigh*" process, but is limited by the back-diffusion of the atmospheric moisture. This was further confirmed by observations on the Tritium balance of lakes (e.g. Oestlund and Berry, 1970) where the vapour exchange between the surface waters and the ambient air could be identified as an important factor.

More quantitative evaluations of the buildup of the heavy isotopes (either of the oxygen or hydrogen isotopes) during evaporation further suggested that the stable isotope fractionation in accompaniment of the evaporation is larger than that to be expected based on the "*Equilibrium Fractionation*" factor. The comparison of the buildup of the Deuterium with that of the Oxygen-18, as shown by Craig (1961), also indicated that the relative rate of change could not be explained by simply applying an *equilibrium fractionation* to the evaporation process. To account for this discrepancy, a number of possible "*transport*" effects were considered, namely: the diffusion of water molecules through the air column, self-diffusion in the liquid or the rates of detachment or attachment of water molecules at the liquid surface. The fact that the diffusion of the water molecules through the air column is an important factor in the isotope fractionation that accompanies the evaporation process was established by comparative laboratory experiments in a nitrogen and helium atmosphere, respectively, as reported by Gat and Craig (1965).

With these findings in mind, and based on the realisation that the rate of evaporation of water into un-saturated air above it is limited by the transport of vapour from the layer immediately above the surface into the ambient atmosphere (Brutsaert, 1965), Craig and Gordon in 1965 suggested the model schematically shown in Fig. 4.2 to describe the isotope fractionation during the evaporation process; it follows the formulation of the *Langmuir linear-resistance* model (Sverdrup, 1951).

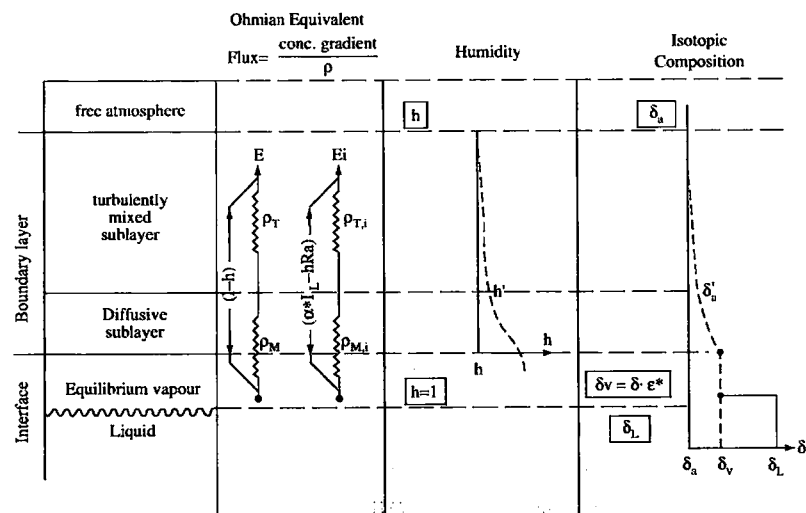


Fig. 4.2. The Craig-Gordon model of isotope fractionation during the evaporation from a free water surface.

The following assumptions underlie this model:

- The establishment of liquid-vapour equilibrium at the water-air interface is rapid compared to the vapour transport from the interface zone to the free ambient atmosphere, so that isotopic equilibrium between the surface water and the saturated vapour above it can be assumed. The (saturation) vapour content and the appropriate isotope fractionation factor thus depends on the temperature and salinity of the water surface (*cf.* Chapter 2 and Box 2.2).
- The vertical flux is the balance between the upward evaporation flux and the back-diffusion of the vapour from the ambient atmosphere.
- No divergence or convergence in the vertical air column.
- No isotope fractionation in accompaniment of fully turbulent transport.

In the model, the vapour flux is described in terms equivalent to *Ohm's Law* as the quotient of the concentration gradient (expressed in terms of the humidity gradient, normalized to a value of $h = 1$ in the saturated surface layer) to the transport resistance, ρ . In the case of a stagnant air layer above the liquid surface, as pertains to the evaporation through an unsaturated soil layer as well as the water loss from plants through the

stomata (to be described below), the vapour transport is by molecular diffusion and a fixed linear concentration profile is established. The water and isotope fluxes through the air column are then determined by their respective molecular diffusion coefficients. On the other hand, for an open interface under strong wind conditions, most of the transport is by turbulent diffusion, and molecular diffusion through a non-steady variable air layer is effective only close to the surface (Brutsaert, 1965).

The appropriate flux equation for the water substance is then as follows:

$$E = (1 - h)/\rho \quad (4.7)$$

where ρ is the sum of the resistance to diffusive flow in the diffusive sublayer, ρ_M , and in the turbulently mixed sub-layer, ρ_T , namely:

$$\rho = \rho_M + \rho_T.$$

The corresponding flux equations for the water isotopologues, either of $^1\text{H}^2\text{HO}$ or H_2^{18}O , are:

$$E_i = (\alpha \cdot R_L - h \cdot R_A)/\rho_i \quad (4.8)$$

where $\rho_i = \rho_{i,M} + \rho_{i,T}$.

The isotope composition of the evaporation flux, R_E , is then the quotient of Eqs. 4.8 and 4.7:

$$R_E = E_i/E = [\alpha \cdot R_L - h \cdot R_A]/[(1 - h) \cdot (\rho_i/\rho)] \quad (4.9)$$

This can be expressed in the more conventional δ -units as follows:

$$\begin{aligned} \delta_E &= [\alpha \cdot \delta_L - h \cdot \delta_A - \epsilon^* - \Delta\epsilon]/[(1 - h) + \Delta\epsilon/10^3] \\ &\approx [\delta_L - h \cdot \delta_A - \epsilon^* - \Delta\epsilon]/(1 - h) \end{aligned}$$

In this expression, $\epsilon^* \equiv (1 - \alpha) \cdot 10^3$ and $\Delta\epsilon \equiv (1 - h) \cdot (\rho_i/\rho - 1) \cdot 10^3$. In the linear resistance model, $\rho_i/\rho = (\rho_{i,M} + \rho_{i,T})/(\rho_M + \rho_T)$ so that the term $(\rho_i/\rho - 1)$ can be written as follows:

$$(\rho_i/\rho - 1) = (\rho_M/\rho) \cdot (\rho_{i,M}/\rho_M - 1) + (\rho_T/\rho) \cdot (\rho_{i,T}/\rho_T - 1).$$

The term $(\rho_{i,T}/\rho_T - 1)$ can be eliminated, based on the model assumption that there is no isotope fractionation during turbulent transport so that $\rho_{i,T} = \rho_T$. Defining $\theta = (\rho_M/\rho)$, the expression for $\Delta\epsilon$ is then:

$$\begin{aligned} \Delta\epsilon &= (1 - h) \cdot (\rho_M/\rho) \cdot (\rho_{i,M}/\rho_M - 1) \cdot 10^3 \\ &= (1 - h) \cdot \theta \cdot (\rho_{i,M}/\rho_M - 1) \cdot 10^3 \end{aligned} \quad (4.10)$$

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In the case of a stagnant diffusion layer, ρ_M is proportional to D^{-1} . Under strong turbulent wind conditions and a rough water surface, the transient-eddy model of Brutsaert (1975) can be applied where ρ_M is proportional to $D^{-1/2}$. For more moderate interface conditions, a transition from the proportionality of $D^{-1/2}$ to D^{-1} occurs (Merlivat and Coantiac, 1975). As described in Chapter 3.2, the ratio of the molecular diffusivities through air of the water isotopologues differ appreciably from those expected from the gas-kinetic theory, based on the molecular weights only. Defining the ratio of the molecular diffusivities of the heavy and light water molecules, respectively, as C_K then, the expression for $\Delta\epsilon$ becomes:

$$\Delta\epsilon = (1 - h) \cdot \theta \cdot n \cdot C_K. \quad (4.10a)$$

The value of n ranges between the value of $n = 1$ for stagnant, and $n = 1/2$ for fully turbulent wind conditions, respectively.

The formulations just presented apply to a well-mixed and homogenous water body where at all times there is no difference of isotope composition between the surface and the bulk of the liquid. This is not necessarily true in the case of open water under wind-still conditions or when evaporation takes place from within the soil. Under such circumstances, a concentration gradient can be established in the liquid boundary layer which slows down the isotopic change that accompanies the evaporation, as elaborated in Box 4.1. The evaporation from within the soil column will be discussed in Chapter 8.

Due to the additional transport-fractionation imposed on the evaporation process under natural conditions, the ratio of change of the Deuterium to Oxygen-18 labeled waters during such a process differs from that of the liquid-vapour transition under equilibrium conditions. On a plot of $^2\delta$ versus $^{18}\delta$ (δ -plot for short), such evaporated waters are then located on "Evaporation Lines", whose slopes are reduced relative to the Equilibrium Lines. These relationships will be further elaborated in the context of the discussion on surface waters in Section 9.4 and Fig. 9.8.

The historical evolution of the concepts leading to the Craig-Gordon Evaporation Model has recently been reviewed by Gat (2008) and the model was re-evaluated by Horita, Rozanski and Cohen (2008).

Box 4.1 The case of incomplete mixing in the liquid surface layer.

The formulations of the Craig-Gordon model shown were based on the assumption that the evaporating water body was well mixed, so that at all times, $\delta_L(\text{surface}) = \delta_L(\text{bulk})$. This is not necessarily so for an open water body under no-wind conditions or for evaporation from within a porous medium, e.g. soils. Incomplete mixing can then result in the enrichment of the surface water in the heavy isotopes relative to the bulk of the liquid and, hence, the establishment of a concentration gradient in the liquid boundary layer. In the Craig-Gordon model, one then has to introduce an additional resistance, ρ_L .

On the assumption of a surface boundary layer of constant depth, the following formulation represents the isotope flux through the boundary layer:

$$E_i = E \cdot R_L - (R_{\text{surface}} - R_L)/\rho_L \quad (1)$$

On adding this expression to the flux equation in the atmospheric boundary layer, where the term R_{surface} substitutes for R_L and disregarding at this stage any change in the values of α^* and ϵ^* due to a temperature or salinity change in the surface layer, then the increase of the isotope composition resulting from the evaporation is given by Eq. 2:

$$d\ln R_L/d\ln N = [h \cdot (R_L - R_a)/R_L - \epsilon^* - \Delta\epsilon]/[(1 - h) + \Delta\epsilon + \alpha^* \cdot E \cdot \rho_L] \quad (2)$$

to be compared to Eq. 3 for the mixed surface waters:

$$d\ln R_L/d\ln N = [h \cdot (R_L - R_a)/R_L - \epsilon^* - \Delta\epsilon]/[(1 - h) + \Delta\epsilon]. \quad (3)$$

Obviously then, when $\rho_L \neq 0$, the isotopic change is slowed down.

4.3.1. Isotope composition of the evaporation flux — δ_E

The isotopic composition of the evaporation flux is usually the least known parameter in the balance equation of the water substance, as it is not directly amenable to measurement. Based on the Craig-Gordon model of evaporation from an open-water body, it is expressed by Eqs. 4.9a and 4.10. For operational use, this can be written in the following form:

$$\delta_E \approx [\delta_L - h \cdot \delta_A - \epsilon^* - (1 - h) \cdot \theta \cdot n \cdot C_K]/[1 - h] \quad (4.11)$$

where $C_K = (\rho_{i,M}/\rho_M - 1) \cdot 10^3$. Based on the measurements of Merlivat (1978) this has the value of $C_K = 28.5(\text{‰})$ in the case of the oxygen-18 labelled water molecule and $C_K = 25.1(\text{‰})$ for the deuterated water molecule diffusing through air; n is the exponent in the relationship between ρ_M and D , which, as discussed above, is usually assumed to be $n = 1/2$ for

evaporation from the ocean. $\theta = (\rho_M/\rho)$; this can be assumed equal to 1 for a small water body whose evaporation flux does not perturb the ambient moisture significantly. However, it was shown to be $\theta = 0.88$ in the case of the North American Great Lakes (Gat *et al.*, 1994) and that a value of $\theta = 0.5$ applies in the eastern Mediterranean Sea (Gat *et al.*, 1996). The latter value appears to be the limiting factor encountered also over large open oceanic areas.

4.3.2. Postscript

As mentioned in Section 3.2, Cappa *et al.* (2003) from recent measurements on the isotope composition of evaporating waters concluded that the main reason for the apparent discrepancy between the expected and measured fractionation due to diffusion of the water vapour from the surface was not due to the Diffusion Constant (D), as given by Merlivat (1978), but due to a lower temperature at the water surface resulting from incomplete mixing of the water in the liquid boundary. This issue is not yet settled as of now, and an authoritative re-evaluation of the isotope fractionation in accompaniment of evaporation is called for.

4.4. The "Isotope Transfer Function" (ITF)

The set of rules that describe the change in isotope composition resulting from the passage of the water substance from input to output of a system is termed the *Isotope Transfer Function* (Gat, 1997). Two effects have to be taken into account namely, on the one hand, those actuated by fractionation between the isotopes in accompaniment of phase transitions or kinetic and transport processes and, on the other hand, when there is a selection of part of the input waters during the transition to the output, provided these selected parcels are characterised by a different isotopic composition. An apparent further change can occur simply by mixing of water parcels of different composition. An example is the change of isotope composition that accompanies the transition of precipitation input through the land/biosphere/atmosphere interface to groundwater recharge flux or surface runoff, as discussed in more detail in Chapters 8 and 9.

Figure 4.3 summarizes the occurrence of these different processes throughout the water cycle.

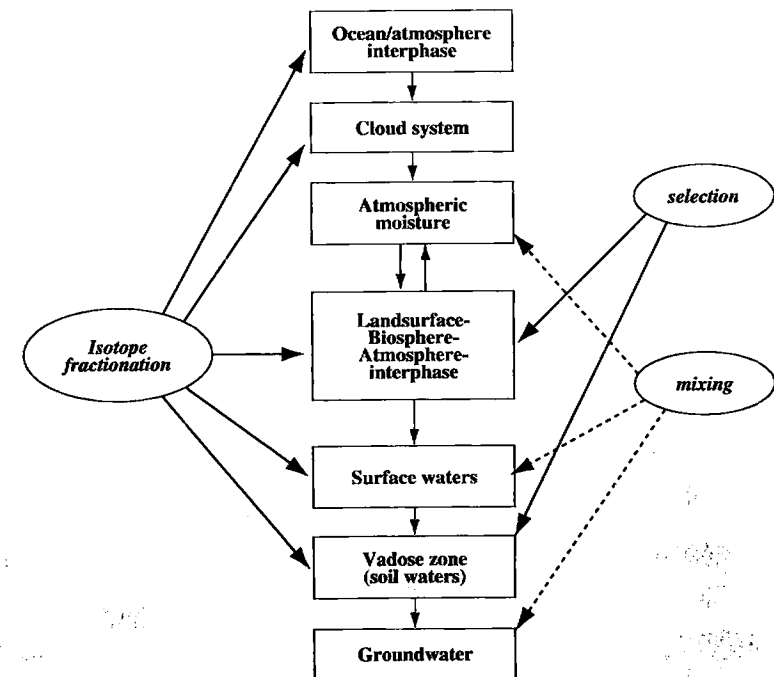


Fig. 4.3. Isotope composition changes along the components of the hydrologic cycles. The diagram indicates the sites where isotope fractionation, selection or mixing processes operate.

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Isotope Effects

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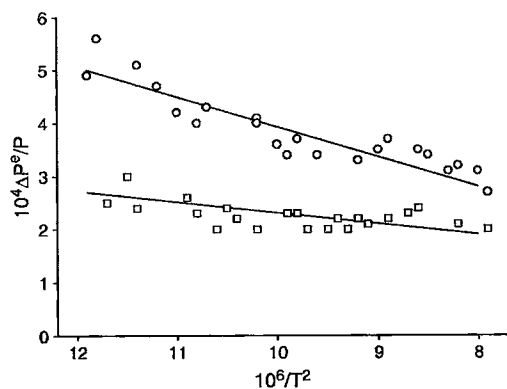


Fig. 5.7 Excess pressures of equimolar solutions of *per*-protio and *per*-deutero benzene and of *per*-protio and *per*-deuterocyclohexane. $\Delta P = (P_{\text{SOLUTION}} - [0.5 P^{\circ}(\text{per-protio}) + 0.5 P^{\circ}(\text{per-deutero})])$. The upper line refers to cyclohexane ($\text{C}_6\text{H}_{12}/\text{C}_6\text{D}_{12}$) solutions, the lower to benzene ($\text{C}_6\text{H}_6/\text{C}_6\text{D}_6$) (Redrawn with permission from Jakli, Gy., Tzias, P., and Van Hook, W. A. *J. Chem. Phys.* **68**, 3177 (1978). Copyright 1978, American Institute of Physics)

fluid [$(0.5 \text{ C}_6\text{H}_6 + 0.5 \text{ C}_6\text{D}_6)$, see Fig. 5.7] in good agreement with experiment, $2.1 \pm 0.3 \text{ J/mol}$. The agreement furnishes strong support for the theoretical development (Section 5.8) correlating molar volume isotope effects and excess free energies of solutions of isotopomers.

5.7.4 Example #4: Water

It is instructive to illustrate the use of Equations 5.19 and 5.29 using a simplistic 3-atom model calculation for water. Even though it is well established that the condensed phase structure of water is complicated and involves the coordinated motions of many molecules coupled through a constantly fluctuating hydrogen bond network, the fundamentals of the VPIE are represented reasonably well by the simplified model.

The principal features of water VPIE's are illustrated in Fig. 5.8 which compares VPIE's for water isotopomers from well down in the solid-vapor part of the phase diagram to near 400 K. For $\text{H}_2\text{O}/\text{D}_2\text{O}$ and $\text{H}_2^{16}\text{O}/\text{H}_2^{18}\text{O}$ high precision data from VPIE or LVFF, respectively, are available all the way to the critical point. VPIE's in the solid, and in the liquid not too far from the melting point, are normal and unusually large. At 273.15 K for $\text{H}_2\text{O}/\text{D}_2\text{O}$, $\ln(P'/P)_{\text{SOLID}} = 0.242$ and $\ln(P'/P)_{\text{LIQ}} = 0.204$. Such large effects are consequent to the large vibrational frequency shifts which occur on the condensation of a freely rotating and translating vapor molecule to the librational hindered and hydrogen-bonded condensed phase. Although the OH/OD stretching modes red shift significantly on condensation, that change is more than compensated by the very large and isotope sensitive blue shift in external frequencies.

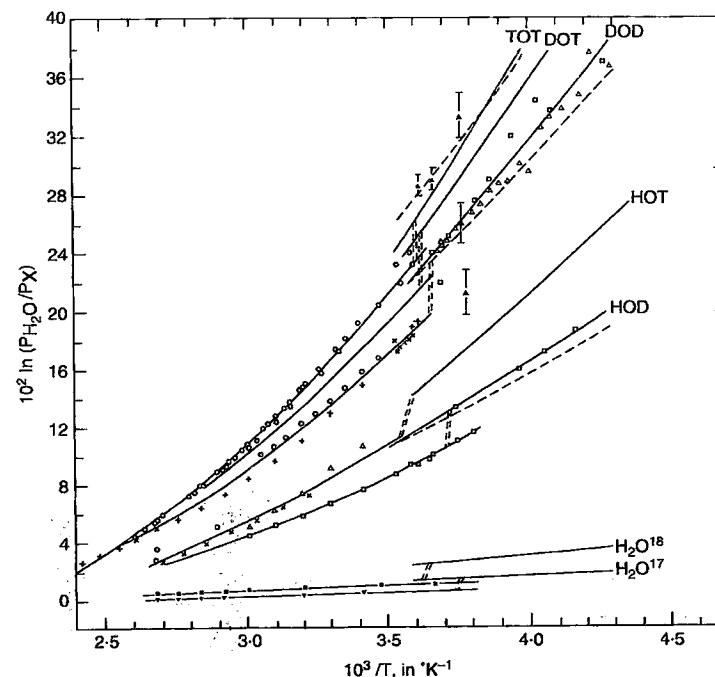


Fig. 5.8 VPIE's of Waters and Ices. The points are experimental from various sources. The lines are calculated using an isotope independent harmonic force field consistent with spectroscopic information (Van Hook, W. A. *J. Phys. Chem.* **72**, 1234 (1968))

The temperature coefficient of VPIE is also unusually large. For $\text{H}_2\text{O}/\text{D}_2\text{O}$ VPIE (normal at low temperature) falls steeply from 0.204 at the triple point to 0.051 at the boiling point, continues to drop as it crosses into the inverse VPIE region at 494 K, and still falls, albeit more slowly, all the way to the critical point of H_2O , 647.2 K, where it is -0.024 . Within experimental error $\ln(\alpha)$ is zero at that temperature. The interpretation of these temperature dependences is straightforwardly connected with the loss of hydrogen bonding as temperature increases. At high enough temperature, H-bonding has essentially disappeared, and the librational frequencies have all but vanished. Near T_c the most important contribution to IE is from the red-shifted OH/OD stretches. Thus, in this region the interpretation is similar to that of a hydrocarbon or other van-der-Waals bonded fluid. Table 5.8a shows observed and calculated frequencies and frequency shifts for room temperature water and VPIE's calculated from a harmonic isotope independent force field (Table 5.8b) chosen to be consistent with the spectroscopic phase frequency shifts

Table 5.8a Gas phase frequencies and observed and calculated gas-ice (173 K) and gas-liquid (213 K) frequency shifts for H₂O and D₂O. Values in cm⁻¹ (Van Hook, W. A. *J. Phys. Chem.* 72, 1234 (1968))

	H ₂ O		D ₂ O	
Gas to solid at 173 K				
ν_{GAS}	$(\nu_{\text{GAS}} - \nu_{\text{ICE}})_{\text{OBS}}$	$(\nu_{\text{GAS}} - \nu_{\text{ICE}})_{\text{CALC}}$	$(\nu_{\text{GAS}} - \nu_{\text{ICE}})_{\text{OBS}}$	$(\nu_{\text{GAS}} - \nu_{\text{ICE}})_{\text{CALC}}$
Internal modes				
3755.8	375.8	375.9	363	368.3
3656.7	506.7	506.5	277	274.8
1594.6	-55.4	-55.4	-31.7	-43.1
Librations ^a	-840	-843.6		-596.8
	-770	-772.9		-557.7
	-660	-666.7		-501.2
Hindered	-250	-275		-261
Translations ^a	to	-275		-260
	-300	-275		-258
Gas to liquid at 313 K				
ν_{GAS}	$(\nu_{\text{GAS}} - \nu_{\text{LIQ}})_{\text{OBS}}$	$(\nu_{\text{GAS}} - \nu_{\text{LIQ}})_{\text{CALC}}$	$(\nu_{\text{GAS}} - \nu_{\text{LIQ}})_{\text{OBS}}$	$(\nu_{\text{GAS}} - \nu_{\text{LIQ}})_{\text{CALC}}$
Internal modes				
3755.8	125.8	125.8	126.1	92
3656.7	206.7	206.6	156.5	150.4
1594.6	-50.4	-50.6	-46.7	-38.0
Librations ^a	-450	-500.0		-371.7
	to	-496.3		-358.0
	-600	-495.4		-353.6
Hindered ^a	-150	-162.2		-153.7
Translations ^a	to	-162.2		-153.5
	-175	-162.2		-154.0

^aBroad spectral features. Approximate center reported.

of H₂O and D₂O. VPIE's for various water isotopomers calculated from this force field are those shown in Fig. 5.8 as the solid lines.

In treating mixtures of isotopes in aqueous systems one must consider disproportionation, H₂O + D₂O = 2 HOD, at the same time recognizing that a pure sample of HOD cannot be obtained in bulk. Careful consideration of data on vapor pressures and freezing and boiling points of H₂O/HOD/D₂O mixtures leads to the conclusion that $\ln [P(\text{H}_2\text{O})/P(\text{D}_2\text{O})] / \ln [P(\text{H}_2\text{O})/P(\text{HOD})] = 1.91 \pm 0.02$ ($273 < T/K < 473$) and is essentially independent of HOD concentration. In first approximation one would expect a ratio of two, which is the value predicted by the law of the geometric mean, but that law does not properly account for the interactions between internal and external modes, large and important in water because of hydrogen bonding.

IE's on some of the other properties of water are shown in Table 5.9. Many properties (like the enthalpies of phase change, triple points, etc.) are closely related to VP and can be interpreted similarly. Molar volume isotope effects are interesting and are discussed in Chapters 12 and 13. In the low temperature liquids

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Table 5.8b Isotope independent gas, liquid and solid force fields for water. Units are mdyn/A for stretches and mdyn — A/rad² for bends (Van Hook, W. A. *J. Phys. Chem.* 72, 1234 (1968))

	Gas	Liquid (313 K)	Solid
OH stretch	8.4543	7.7470 ^a	6.6435
HOH bend	0.6994	0.7448 ^a	0.7520
Stretch-stretch	-0.1002	-0.2705	-0.3577
Stretch-bend	0.2272	0.2371	0.2371
Stretch-libration		-0.0234	-0.0480
Bend-libration		0.0072	0.0086
Translations		0.2789 ^a	0.8020
Libration 1		0.0896 ^a	0.1625
Libration 2		0.1705 ^a	0.4858
Libration 3		0.2575 ^a	0.6249

^aThese constants are temperature dependent. $df(\text{st}) = 2.8 \times 10^{-3}$ mdyn/(A deg) equivalent to the observed $d\nu(\text{OH})/dT = 0.696 \text{ cm}^{-1}/\text{deg}$; $df(\text{bend}) = -0.052 \times 10^{-3}$ mdyn A/(rad² deg) equivalent to the observed $d\nu(\text{HOH})/dT = -0.061 \text{ cm}^{-1}/\text{deg}$. For the external librations and translations $df(\text{ext})/f(\text{ext}) = -0.0041 T + 1.2839$

Table 5.9 IE's on some of the thermodynamic properties of isotopic waters (Jancso, G. and Van Hook, W. A. *Chem. Rev.* 74, 689 (1974))

	HOH	DOD	TOT	H ₂ ¹⁸ O	H ₂ ¹⁷ O
$t(\text{triple})/^\circ\text{C}$	0.0	3.82	4.49	0.38	0.21
$t(\text{boil}) - 100/^\circ\text{C}$	0.0	1.42	1.51	0.15	0.08
VPIE crossover (c.o.)/ ^o C	—	220.9	190	no c.o.	no c.o.
Critical properties					
T_c'/K	647.1				
$\ln(T_c'/T_c)$		0.0050			
P_c'/MPa	22.06				
$\ln(P_c'/P_c)$		0.0180			
$\rho_c'/(\text{mol}/\text{m}^3)$	17.9				
$\ln(\rho_c'/\rho_c)$		0.0056			
Relative enthalpy ($L_x - L_{\text{H}_2\text{O}}$)					
0 ^o C (J mol ⁻¹)	—	1750	1960	67	38
100 ^o C (J mol ⁻¹)	—	810	1210	46	25
δH_{fus} (J mol ⁻¹), 0 ^o C	6010	5960			
(J mol ⁻¹), 3.82 ^o C	—	6280			
C_p (J mol ⁻¹ K ⁻¹), 25 ^o C	75.3	84.3			
C_v (J mol ⁻¹ K ⁻¹), 25 ^o C	74.5	83.7			
$10^6 \kappa_T/\text{atm}^{-1}$, 25 ^o C	45.3	46.6			

molar volumes are in the order TOT > DOD > HOH > HH¹⁸O. These inverse isotope effects have been ascribed to librational motion in the hydrogen bonded condensed phase. The center of mass and center of interaction (about which the molecule librates) do not coincide and translation-rotation coupling must be taken into account. The center of mass moves progressively away from the oxygen in the

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series ^{18}OHH , ^{16}OHH , ^{16}ODD , ^{16}OTT and this explains the ordering in the low temperature liquids. At higher temperatures MVIE falls off to more negative values due to ever increasing isothermal compressibility (unbounded at T_C). Near T_C this fixes the order of isobaric molar volumes as $\text{TTO} > \text{DDO} > \text{HH}^{18}\text{O} > \text{HH}^{16}\text{O}$ and the ordering of critical temperatures as $\text{HH}^{16}\text{O} > \text{DDO} > \text{TTO}$.

Numerous model calculations correlating aqueous VPIE's using simple harmonic or *pseudo*-harmonic cell models have been reported (see Fig. 5.8 and Table 5.8 for an ultra-simple version). Such calculations show the importance of the librational hydrogen bonded modes and the stretch-libration interaction in determining VPIE for D or T substitution.

5.8 Excess Free Energies in Solutions of Isotopes: Connections Between VPIE, the Liquid Vapor Fractionation Factor, α , and RPFR

The pseudo-harmonic cell model leads straightforwardly to the idea that the excess free energies of isotopomer mixtures correlate with the isotope effect on the molar volumes of the pure isotopomers (MVIE's). An excess thermodynamic property, Y^E is just the difference between the value of that property for the real (nonideal) solution and the corresponding ideal solution, $Y^E = Y - Y^{\text{ideal}}$. MVIE's are discussed in Chapters 12 and 13. We begin by expanding the Helmholtz free energy about the equilibrium volumes, V^{or} or V° , to obtain an expression for the contribution of ΔV to the excess Helmholtz free energy

$$A^E(V) = x x' (V' - V) [(dA/dV) - (dA'/dV)] + (1/2) x x' (V' - V)^2 [x (d^2A/dV^2) + x' (d^2A'/dV^2)] + \dots \quad (5.40)$$

Here x and x' are isotopomer mole fractions in the binary mixture. Remembering $x' = 1 - x$, differentiating to obtain partial molar free energies (and using the thermodynamic relations $\mu^E(V) = A^E(V) - x' (dA^E(V)/dx')$ and $\mu^{E'}(V) = A^E(V) + x' (dA^E(V)/dx')$ one finds expressions for the excess partial molar free energies, $\mu^E(V)$ and $\mu^{E'}(V)$. In the high dilution limit, an important case of practical interest, the excess chemical potential of the trace isotopomer, say the unprimed one, is

$$\mu^{\infty,E} = (V^{\text{or}} - V^{\circ}) [(dA/dV) - (dA'/dV)] + (1/2) (V^{\text{or}} - V^{\circ})^2 [(d^2A'/dV^2)] : x' \rightarrow 1 \quad (5.41)$$

The development to this point has been rigorous but difficulties arise as simplifications are introduced. Early authors continued by discarding the first term in Equation 5.40 since $V^{\circ} \sim V^{\text{or}}$ and $[(dA/dV)_T = P \sim (dA'/dV)_T = P']$, and identified $(d^2A/dV^2)_T$ with $1/(\kappa V)$, κ the isothermal compressibility. Then $\mu^{\infty,E} = (V^{\text{or}} - V^{\circ})^2 / (2\kappa V^{\circ})$. Since $\text{RPFR} = -\delta\Delta A^{\circ}/RT$ and RT

Equation 11.57 signifies that when the competitive method is used (i.e., both isotopomers are present simultaneously in the reaction mixture) the experimentally determined kinetic isotope effect corresponds to the isotope effect on V/K regardless of the actual concentration of the substrate. In other words, one cannot measure the isotope effect on V_{max} using this method even when concentration is much larger than the Michaelis constant K_M .

11.4 Aqueous Solvent Equilibrium and Kinetic Isotope Effects

It is reasonable to expect that isotopic substitution on solvent molecules will affect both equilibrium and rate constants. This is especially true for reactions in aqueous media, many of which are acid or base catalyzed and therefore sensitive to pH or pD. Furthermore H/D aqueous solvent isotope effects often display significant nonlinearity when plotted against isotope fraction of the solvent. The analysis of this effect can yield mechanistic information. The study of aqueous solvent isotope effects is particularly important in enzyme chemistry because enzyme reactions universally occur in aqueous media and are generally pH sensitive.

11.4.1 $\text{H}_2\text{O}/\text{D}_2\text{O}$ Solvent Effects on pH (pD) and pK_a

The isotope effect on the acid dissociation constants for H_2O and D_2O has been carefully measured by many workers, most notably Paabo and Bates working at the US Bureau of Standards. Comparing the reactions $2 \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{OH}^-$ and $2 \text{D}_2\text{O} \rightleftharpoons \text{D}_3\text{O}^+ + \text{OD}^-$ they found

$$\Delta pK_a = -\log[(\text{D}_3\text{O}^+)(\text{OD}^-)] - (-\log[(\text{H}_3\text{O}^+)(\text{OH}^-)]) = 0.958 \quad (11.58)$$

In other words the acid dissociation constant of ordinary water, $K_a(\text{H}_2\text{O}) = 1 \times 10^{-14}$, is about ten times larger than that of heavy water, $K_a(\text{D}_2\text{O}) = 1.10 \times 10^{-15}$. Thus the pH of ordinary water is $\text{pH} = -\log((\text{H}_3\text{O}^+)) = -\log[(1 \times 10^{-14})^{1/2}] = 7.00$ while pD for D_2O is 7.48. This is a significantly large isotope effect and has important consequences.

It is interesting that a plot of ΔpK_a 's vs. $pK_a(\text{H}_2\text{O})$ for a series of weak inorganic acids $\text{D}_3\text{PO}_4/\text{H}_3\text{PO}_4$, $\text{D}_2\text{PO}_4^-/\text{D}_3\text{PO}_4^-$, $\text{DCO}_3^-/\text{HCO}_3^-$, $\text{D}_2\text{O}/\text{H}_2\text{O}$ shows an excellent straight line correlation. This lends credence to the numerical value quoted in Equation 11.58. That correlation is shown in Fig. 11.4. On the other hand, ΔpK_a 's measured on a wide variety of organic acids scatter widely (Fig. 11.4). The correlation for these organic acids is not well established (see the caption to the figure) and many authors choose to approximate the effect by taking the average. For the ~ 100

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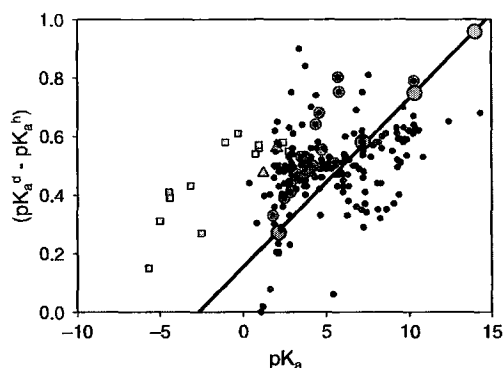


Fig. 11.4 Isotope effect on acid ionization constants. The heavy line through the large circles correlates data on inorganic weak acids (D_3PO_4 , D_2PO_4^- , DCO_3^- , D_2O) due to Paabo, M. and Bates, R. C. (*J. Phys. Chem.* 74, 706 (1970)), $\Delta\text{pK}_a = 0.1537 + 0.0577\text{pK}_a$, $r^2 = 0.9994$. It yields $\Delta\text{pK}_a(\text{D}_2\text{O}) = 0.961$ and $\text{pD}(\text{D}_2\text{O}) = 7.481$. The smaller data points refer to ionization of numerous weak organic acids and have been measured or collected by Li, N. C., Tang, P. and Mathur, R. (mid-size circles, *J. Phys. Chem.* 65, 1074 (1961)), Hogfeldt, E. and Bigeleisen, J. (squares, *J. Am. Chem. Soc.* 82, 15 (1960)), Lowe, B. M. and coworkers (triangles, *J. Chem. Soc. Trans. Far. Soc.* 69, 1934 (1973); 70, 362 (1974); 71, 389 (1975)), or Laughton, P. M. and Robertson, R. E. (dots, *Solute-Solvent Interactions*, Coetzee, J. F. and Ritchie, C. D., Eds., Marcel Dekker, NY, 1969). The correlation through these data (not shown), $\Delta\text{pK}_a = 0.40 + 0.02\text{pK}_a$, $r^2 = 0.239$, is not reliable. Most authors prefer a simple average $\langle\Delta\text{pK}_a\rangle = (0.56 \pm 0.14)$.

organic acids plotted in Fig. 11.4 with pK_a 's between 4 and 9, $\langle\Delta\text{pK}_a\rangle = 0.5 \pm 0.1$, i.e. on average the deuterated organic acids dissolved in D_2O are only about 30% as dissociated as their protiated sister compounds dissolved in H_2O .

11.4.1.1 An Important Consequence

Many rate constants in aqueous solutions are pH or pD sensitive. In particular, enzyme catalyzed reactions often show maxima in plots of pH(pD) vs. rate. The example in Fig. 11.5 is constructed for a reaction with a true isotope effect, $k_H/k_D = 2$, and with maxima in the pH(pD)/rate dependences as shown by the bell shaped curves. These behaviors are typical for enzyme catalyzed reactions. When the isotope effect is obtained (incorrectly) by comparing rates at equal pH and pD, the values plotted along the steep dashed curve result. If, however, the rate constants at corresponding pH and pD ($\text{pD} = \text{pH} + 0.5$) are employed, a constant and correct value is obtained, $k_H/k_D = 2$. Thus for accurate measurements of the isotope effects one must control pH and pD at appropriate values ($\text{pD} = \text{pH} + 0.5$ in our example) using a series of buffers. In proton inventory experiments (see below) buffers should be employed to insure equivalent acidities across the entire range of solvent isotope concentration ($0 < x_D < 1$), x_D is the atom fraction of deuterium $[\text{D}]/([\text{H}] + [\text{D}])$.

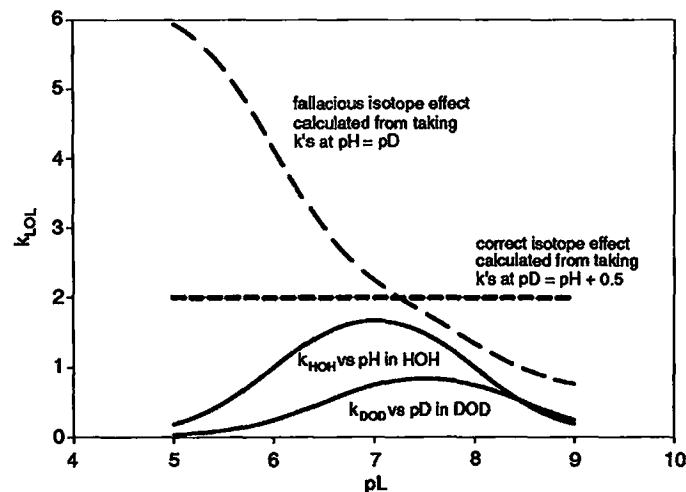
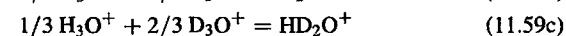
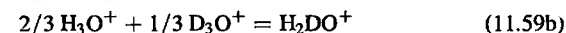


Fig. 11.5 Rate constants in HOH and DOD as a function of pL (two bottom bell shaped curves) for an enzyme catalyzed reaction with three protomeric forms and an isotope effect of 2. L is the H^+ or D^+ concentration as appropriate. The steep curve shows the erroneously calculated isotope effect from the rate constants at $\text{pH} = \text{pD}$. The correct flat line is calculated taking the IE's at corresponding pL ($\text{pD} = \text{pH} + 0.5$) (Schowen, R. L. *J. Label Compd Radiopharm.* 50, 1052 (2007), with permission Wiley Interscience)

11.4.2 Monoprotic Acid/Base Equilibria in Mixed Solvents

In the discussion which follows it is convenient to have a symbol to represent any one of the three hydrogen isotopes, H, D or T, and L (lyonium or lyate) is widely used for that purpose. Thus in a mixture of H_2O and D_2O the solvent L_2O consists of a mixture of H_2O , D_2O and HDO , and the ion L_3O^+ , could be either H_3O^+ , H_2DO^+ , HD_2O^+ , or D_3O^+ , while OL^- represents the two species OH^- and OD^- .



The isotopic abundance of deuterium in the L_3O^+ ion will differ from that in the water with which it is equilibrated. This is expressed in terms of the fractionation factor l which is the ratio of D/H ratios in the lyonium ion and the mixed solvent (water)

$$l = (\text{D}/\text{H})_{\text{L}_3\text{O}^+} / (\text{D}/\text{H})_{\text{L}_2\text{O}} = F_{\text{L}_3\text{O}^+} (1 - x) / [(1 - F)_{\text{L}_3\text{O}^+} x] \quad (11.60)$$

In this equation $F_{L_3O^+}$ is the fraction D in L_3O^+ and x the fraction D in the solvent.

$$x = ([D_2O] + [HDO])/2 / ([D_2O] + [HDO] + [H_2O]) \quad (11.61)$$

$$F_{L_3O^+} = ([D_3O^+] + 2[HD_2O^+]/3 + [H_2DO^+]/3) / ([D_3O^+] + [HD_2O^+] + [H_2DO^+] + [H_3O^+]) \quad (11.62)$$

The square brackets denote number of moles. With the use of the law of the geometric mean, which in this context implies that the equilibrium constants for reactions 11.59a through c are 4, 9/2 and 9/2 respectively, the mixed isotopomer species can be eliminated and Equation 11.60 reduces to

$$l = ([D_3O^+]/[H_3O^+])^{1/3} ([H_2O]/[D_2O])^{1/2} \quad (11.63)$$

While not exact, K_{EQ} for reaction 11.59a is actually 3.8 at 298 K, the approximation is sufficiently accurate for our purposes. With this use of the law of the geometric mean a good deal of algebraic complexity is avoided. Notice that l^6 is the equilibrium constant for the reaction $2H_3O^+ + 3D_2O = 3H_2O + 2D_3O^+$.

Similar fractionation factors can be defined for other species. These are ordinarily given the symbol " ϕ " in order to reserve " l " for solvent fractionation. Thus, for an acid dissociation in mixed media, $AL_j + L_2O = L_3O^+ + AL_{j-1}^-$

$$\phi_{ALj} = F_{ALj}(1-x)/((1-F_{ALj})x) \quad (11.64)$$

For the case of an acid AL with a single exchangeable hydrogen Equation 11.64 reduces to

$$\phi_{AL} = [AD](1-x)/([AH]x) \quad (11.65)$$

Since the ratio of the acid dissociation constants of a monoprotic acid in pure H_2O to that in the mixed solvent is

$$K^H/K^x = [H_3O^+][AL]/[L_2O]/([L_3O^+][AH][H_2O]) \quad (11.66)$$

we can use Equations 11.59 through 11.64 to deduce

$$K^H/K^x = (1-x+x\phi_{AL})/(1-x+xl)^3 \quad (11.67)$$

For pure D_2O ($x = 1$), $K^H/K^D = \phi_{AL}/l^3$. This allows Equation 11.67 to be more usefully written as

$$K^H/K^x = (1-x+x l^3 K^H/K^D)/(1-x+xl)^3 \quad (11.68)$$

The value of l has been determined by NMR measurements on the mixed solvent as 0.69 ± 0.02 and is not to be interpreted as an independent fitting parameter. With l established, ϕ can be calculated from the acid dissociation constants in pure H_2O and D_2O , and Equation 11.68 interpreted as a zero parameter theoretical prediction of

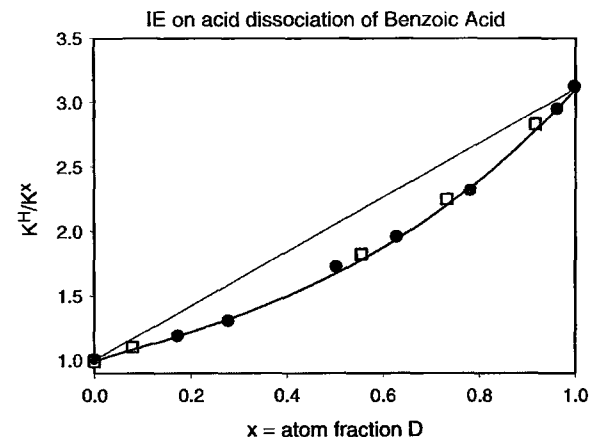


Fig. 11.6 The ratio of acid dissociation constant for benzoic acid C_6H_5COOL as a function of D atom fraction in the solvent. The data are those of Lowe, B. M. and Smith, D. G., *J. Chem. Soc.; Faraday Trans. 71*, 389 (1975) as determined by EMF (circles) and conductance (squares). The heavy line is the prediction of Equation 11.61 with $l = 0.69$, $\phi = 1.02$ and $pK^H = 4.201$. The lighter line shows the linear approximation.

IE's in mixed solvents. The success of the theory is illustrated in Fig. 11.6 using the case of benzoic acid. The agreement between theory and experiment is quantitative.

11.4.2.1 Extension to Dissociation of Polyprotic Acids

For polyprotic acids, $AL_j + L_2O = L_3O^+ + AL_{j-1}^-$, an analogous treatment to the leading to Equation 11.67 gives

$$K^H/K^x = (1-x+x\phi_{ALj})^j / ((1-x+xl)^3 (1-x+x\phi_{AL(j-1)})^{j-1}) \quad (11.69)$$

In pure D_2O ($x = 1$) this becomes $K^H/K^D = \phi_{ALj}^j / (l^3 \phi_{AL(j-1)}^{j-1})$. Consequently the observed value of K^H/K^D can only be used to eliminate one, not both, of the fractionation factors. The other must be considered as an adjustable parameter. That accepted, the available experimental results are consistent with Equation 11.69.

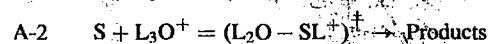
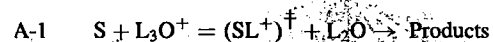
11.4.3 Kinetic Isotope Effects in Mixed Solvents

To calculate the mixed solvent isotope effect on rate constants one applies simple ideas from transition state theory to evaluate the isotope effect on the



[Historical Vignette 11.1] Victor Gold (1922–1985) was born in Vienna but shortly after Hitler's Anschluss emigrated to Britain. He was educated at University and Kings Colleges, London, receiving his graduate degree in 1943. He joined the faculty at Kings in 1944 where he spent his entire career, eventually rising to Head of Department and Dean of Faculty. Gold's most important contribution to isotope science was his development of the field of kinetic solvent isotope effects. He presented generalized expressions for reactions in mixed ($\text{H}_2\text{O}/\text{HOD}/\text{D}_2\text{O}$) solvents and brought forward important ideas on isotope effects in acid/base reactions. (Pencil sketch courtesy of N. Van Hook, 2009)

equilibrium constant between reactant (substrate) and transition state. Several different mechanisms are possible:



For the A-1 mechanism the transition state involves only one hydrogen and the ratio of rate constants follows analogously from Equation (11.67)

$$k^{\text{H}}/k^{\text{x}} = (1 - x + xI)^3 / (1 - x + x\phi^\ddagger) \quad (11.70)$$

where k^{H} is the rate in pure H_2O and k^{x} that in the mixed solvent. Notice Equation 11.70 is similar to the reciprocal of Equation 11.67. That is because the hydronium ion is now being considered as a reactant rather than a product. The single proton fractionation factor for the transition state $\phi^\ddagger$ can be obtained from the observed rate in pure D_2O , $k^{\text{H}}/k^{\text{D}} = I^3/\phi^\ddagger$. On the other hand if there are multiple hydrogens involved in the transition state the rate ratios are deduced from Equation 11.69. For two protons participating in the transition state

$$k^{\text{H}}/k^{\text{x}} = (1 - x + xI)^3 / \left((1 - x + x\phi_1^\ddagger) (1 - x + x\phi_2^\ddagger) \right) \quad (11.71)$$

where the subscript "1" and "2" refer to the fractionation factors for the two transition state protons. Again, one, but only one, of these may be eliminated by using the observed value of $k^{\text{H}}/k^{\text{D}}$. Extension to multiple protons participating in the transition state is straightforward.

It is obvious from the discussion that one can take advantage of equations of the type (11.69), and its generalization to multiple sites, to deduce information about the number of protons actively participating in the transition state, see Fig. 11.7.

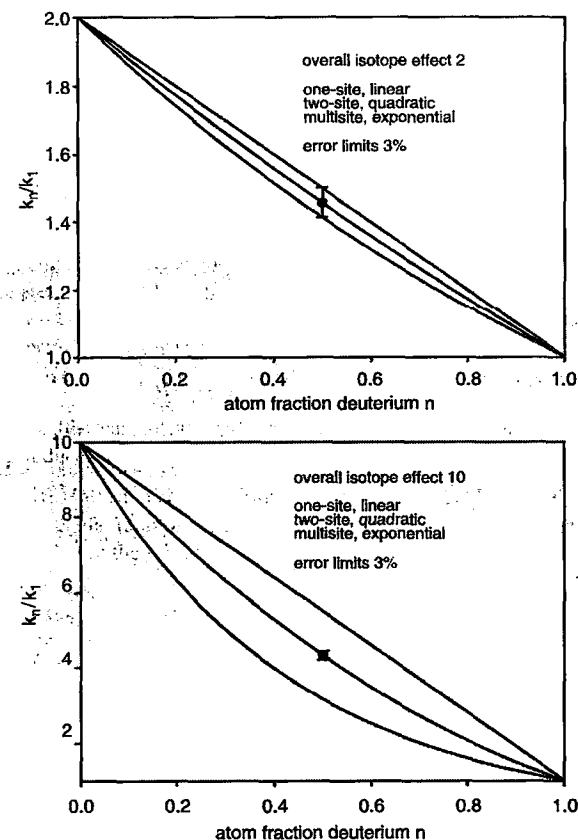


Fig. 11.7 Proton inventory curves (plots of $k(n)/k(\text{H})$ vs. n (or x) = atom fraction D) for overall isotope effects of 2 (*upper plot*) and 10 (*lower plot*). In each, the three curves (reading from *top to bottom*) are for single site, two site, and multi-site isotope effects. Error bars of $\pm 3\%$ (*middle curves*) are shown in each case. For $k_0/k_1 = 2$ the technique is unable to distinguish between the curves at this level of precision (3%), but is more than adequate for $k_0/k_1 = 10$ (Schowen, R. L., *J. Label Compd Radiopharm.* **50**, 1052 (2007), with permission Wiley Interscience)

Isotopes in the Water Cycle

Past, Present and Future of a Developing Science

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4. STABLE OXYGEN AND HYDROGEN ISOTOPES IN PRECIPITATION

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1. Introduction

The fact that water on earth moves in a cycle, from oceans to clouds to rivers to oceans, has been known since ancient civilizations existed on different parts of the globe. However, the quantification of fluxes between various parts of the cycle, particularly the atmospheric and surface parts, has been difficult to achieve even today. Understanding of climatic processes controlling these fluxes and, therefore, the ability to predict variability and changes in the hydrologic cycle, also depends upon the quantification of moisture fluxes between atmospheric and surface reservoirs.

Soon after the discovery of the heavy isotopes of oxygen and hydrogen in the 1920s and early 1930s, significant differences were observed in the isotopic contents of seawater, freshwater, and snow (Gilfillan, 1934; and review by Rankama, 1954). By the early 1950s, as a result of the availability of better measurement techniques, the dependence of isotope content of rain on a variety of climatic factors such as surface air temperature, amount of rain, and the altitude and latitude of precipitation, were also observed (Friedman, 1953; Dansgaard, 1953; Epstein, 1956). Together with the known temperature dependence of isotope ratios (Urey, 1947), the observed relationship between isotopes and climate also provided a means to study past climates by using isotope distributions in continental and marine archives (Emiliani, 1955).

The foundations for the use of isotopes to help quantify fluxes in the hydrologic cycle were firmly established by the 1950s, albeit with a limited scope and limited records of measurements (Craig, 1961; Dansgaard, 1964). Atmospheric nuclear testing of the 1950s and consequent requirements to monitor the tritium fallout resulted in an exponential increase in the number and geographic distribution of stable isotope measurements in the hydrologic cycle (Dansgaard, 1964; Ericsson, 1965; Froehlich et al., this volume). In this summary review, we discuss the developments in stable oxygen and hydrogen isotope measurement techniques, the nomenclature of isotope measurements, the structure of the IAEA/WMO global

network of isotopes in precipitation (GNIP), and an analysis of GNIP data from 1961 to 2000.

2. Analytical Methods

2.1. MEASUREMENT TECHNIQUES

Stable isotope compositions in natural waters are typically measured using double-focusing magnetic sector field mass spectrometers, as originally designed in 1947 (Nier, 1947). Due to the low abundance of isotope species of interest (e.g., ^{18}O - 0.2%) and the very small deviations of isotope ratios (in most cases few per mil only), the precision of such measurements is limited by the stability of mass spectrometers in the time span when multiple samples are analysed. To overcome these limitations, McKinney et al. (1950) designed a dual sample inlet system where a sample and a reference standard were measured in rapid succession. This allowed the measurement of sample isotope ratios with respect to a reference material, resulting in precise measurement of isotope ratios in natural materials. Excellent reviews of mass spectrometric measurement of isotope ratios are given in Gonfiantini (1981) and Platzner (1997).

The basic design of instruments as described above is still used for mass spectrometric measurements today. Continuous flow techniques are now being used by an increasing number of laboratories where tiny sample aliquots are introduced in sample preparation lines (e.g. high temperature pyrolysis oven for oxygen isotope analysis) and flushing the reaction product (e.g. CO gas) with a continuous stream of helium carrier gas for mass spectrometric analysis. These continuous flow techniques permit a very fast measurement cycle and the possibility of using very small samples. Details on the measurements are given in a number of publications; see e.g. articles in IAEA (2001), Werner and Brand (2001), Gehre and Strauch (2003), Werner (2003).

By measuring isotope ratios in a sample with respect to a common standard, it is not necessary to know all corrections for instrumental effects and interferences in a mass spectrometric measurement. Most of the influences on a sample also affect the reference standard and, therefore, cancel out. As a result, it is more convenient not to report the $^{18}\text{O}/^{16}\text{O}$ ratio (R) of a sample directly, but to report the deviation δ of this ratio from that of the reference standard. Due to the small values of the δ -value commonly all data are multiplied with thousand and reported as per mil deviation:

$$\delta = \frac{R_{\text{sample}} - R_{\text{reference}}}{R_{\text{reference}}} \times 1000 \text{ ‰} \quad (1)$$

A detailed and comprehensive review of all aspects of the δ notation and the realization of a conventional reporting scale is given in Gonfiantini (1981).

2.2. ISOTOPE STANDARDS

The primary criterion for a reference standard is that it should represent a major pool of the element under consideration. By 1953 "average ocean water" was suggested and used as a reference point for isotope-ratio measurements. Because no "average ocean water" existed, Craig (1961) refined this concept by defining the hypothetical Standard Mean Ocean Water (SMOW) as zero-point of that conventional scale in terms of real reference water. The isotope ratios of SMOW were compiled by taking a weighted average of the isotope ratio measurements of different seawater compartments available at that time (Horibe and Kobayakawa, 1960). But because SMOW was just the concept of hypothetical water and never existed as a real water sample, it could not be used directly for calibration of laboratory measurements. Its isotopic composition was defined in terms of an isotopic difference from an existing water sample (NBS 1) distributed by the US National Bureau of Standards (Mohler, 1960). During an IAEA interlaboratory comparison in 1965, serious doubts were raised concerning the preservation of the NBS-1 water standard and about possible changes of its isotopic composition over time. For this reason, it was unsuitable to be used as a standard any more.

An IAEA panel of experts recommended in 1966 that two new primary water reference materials should be established - one being as close as possible to the defined SMOW and the other with an abundance of the heavier isotopes close to the lowest limits observed in natural waters. The new "SMOW" was subsequently prepared by R. Weiss and H. Craig at the Scripps Institution of Oceanography, La Jolla, USA, by mixing distilled ocean water with small amounts of other waters. The second water reference material was obtained by E. Picciotto, Université Libre de Bruxelles, Belgium, from melting a firm sample at Plateau Station, Antarctica. This material was named Standard Light Antarctic Precipitation (SLAP). Control analyses performed by H. Craig indicated that the new "SMOW" had the same $^{18}\text{O}/^{16}\text{O}$ ratio as the defined SMOW, but a slightly lower $^2\text{H}/^1\text{H}$ ratio or $\delta^2\text{H}$ value (-0.2‰) relative to SMOW. This difference, however, is about a factor of four to five lower than the measurement uncertainty of most laboratories and for practical purposes, the two standards had identical isotope ratios. Absolute isotope ratios ($^{18}\text{O}/^{16}\text{O}$, for $^{17}\text{O}/^{16}\text{O}$ as well as for $^2\text{H}/^1\text{H}$) in the standard prepared by Weiss and Craig have recently been determined and are listed in Groening (2004).

Unfortunately the same term, SMOW, was used for reporting isotope measurements using the synthetic water standard. The IAEA then decided to rename the water prepared by Weiss and Craig to VSMOW (Vienna-SMOW) in order to distinguish between the hypothetical SMOW of Craig (1961) and the synthetically prepared standard. An IAEA Consultants' Meeting in 1976 recommended that all future results for hydrogen and oxygen isotope ratios should be reported as δ values relative to VSMOW. To increase coherence and consistency between reported δ values, the meeting also recommended that a fixed δ value for the second water reference material, SLAP, should be adopted for both the $^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/^1\text{H}$ δ and that these values should be normalized relative to VSMOW water, the primary reference material.

The definition of VSMOW as zero-point for the oxygen and hydrogen δ scales and the adoption of fixed δ values for SLAP is therefore a slight modification of the original definition of the δ scale (Coplen et al. 1996):

$$\delta = \frac{R_{\text{SAMPLE}} - R_{\text{VSMOW}}}{R_{\text{VSMOW}}} \left(\delta_{\text{SLAP}} / \frac{R_{\text{SLAP}} - R_{\text{VSMOW}}}{R_{\text{VSMOW}}} \right) \times 1000 \text{ ‰} \quad (2)$$

Due to the limited amount of VSMOW and SLAP produced in the sixties, their stock will be exhausted by about 2010. The IAEA has therefore taken steps to produce replacements for both reference materials. A large quantity (~ 300 litres) of replacement material has been prepared for VSMOW. The new material has an isotopic composition very close to the existing VSMOW and calibration tests reveal deviations in the order of less than 0.01 ‰ for $\delta^{18}\text{O}$ and of about 0.1 ‰ for $\delta^2\text{H}$. Work is in progress to prepare a similar replacement for SLAP.

3. Global Network of Isotopes in Precipitation (GNIP)

3.1. NETWORK STRUCTURE

A panel of experts convened by the IAEA in 1959 recommended a set of network stations to monitor the isotope contents in precipitation. These stations were to form the global network of isotopes in precipitation (GNIP). It was also recommended that monthly composite samples of precipitation would be the basis for isotope monitoring. The network began with 151 precipitation stations at the onset of its operation in 1961, and quickly reached a maximum of about 220 stations in 1963-1964. After a review of the 1961-1975 data, primarily based on the decay of the bomb-derived tritium peak in precipitation, a number of GNIP stations was discontinued in the late 1970s and resulted in a significant loss of global coverage for both tritium and stable isotopes. Following the recognition of the role of precipitation stable isotopes in better simulating the hydrologic cycle in climate models (Jouzel et al., 1984), there was a renewed interest in GNIP data. This led to the re-activation of some of the GNIP stations that had been inactive since the 1970s. A growing water resources programme of the IAEA in the 1980s and 1990s also made it possible to establish or strengthen national networks of isotopes in precipitation and bring them into GNIP. Today, 183 stations are contributing monthly and/or daily samples from 53 countries (Fig. 1).

At the very beginning of GNIP, a few laboratories in the United States of America and Europe (primarily Dansgaard's laboratory in Denmark) analysed most of the GNIP samples. Presently, about 30% of the GNIP samples are analysed at the IAEA laboratory in Vienna (from stations listed as IAEA stations in Fig. 1) and the rest by other laboratories in various countries. These laboratories report the results to IAEA once a year for their incorporation into the GNIP database. To date, a total of 70 laboratories from 38 countries have contributed to the GNIP database. National laboratories providing GNIP data participate in inter-laboratory comparisons organized by the IAEA on a regular basis, thus maintaining the quality of analytical data reported as a part of GNIP.

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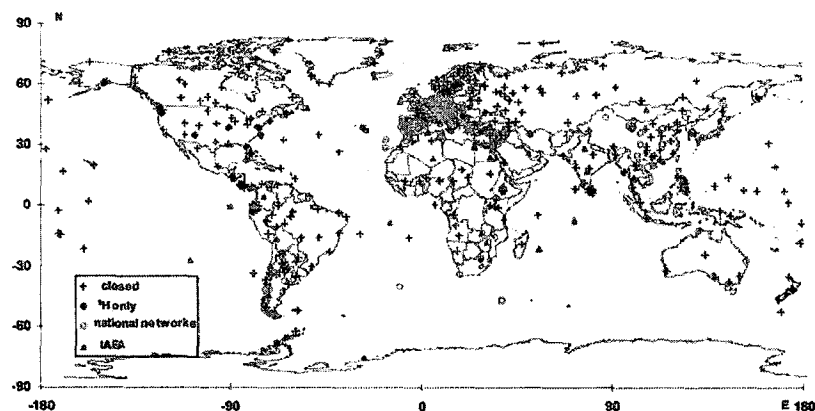
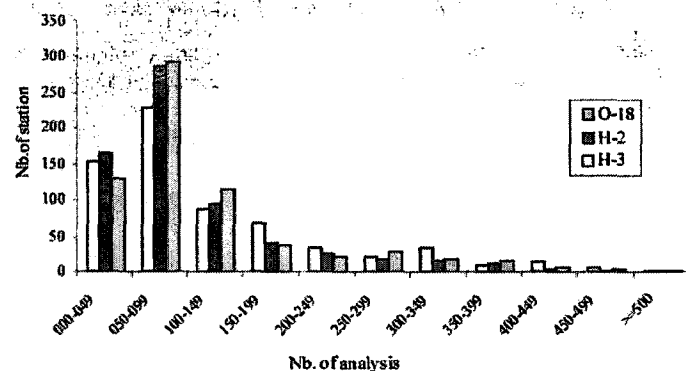


FIG. 1. Global Network of Isotopes in Precipitation in 2003.

3.2. DATABASE

The GNIP database is located at the IAEA Headquarters in Vienna (<http://isohis.iaea.org>) and allows easy access to data through numerous selection criteria. More than 88,000 records are available and can be downloaded freely. The longest isotope record is available for the station in Ottawa, Canada, where tritium in precipitation has been measured since 1954. For 79 stations, less than 12 months of isotope and meteorological data are available in the database. For a majority of stations, the length of isotope records varies between 4 and 8 years (Fig.2).

FIG. 2. Numbers of ^{18}O , ^2H , ^3H records in the GNIP database.

The spatial distribution of data accumulated in the GNIP database is far from homogeneous owing to difficulties in maintaining stations at high altitudes, high latitudes and/or isolated locations such as small islands. As can be seen in Fig.3, 52% of GNIP stations are located within the $30^\circ - 60^\circ \text{N}$ latitude band, and 72% of the stations are located at altitudes between 0 and 500 m.

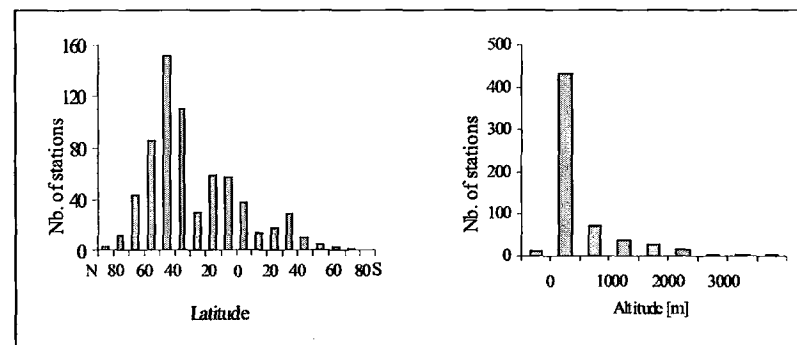


FIG. 3. Latitude and altitude distribution of the GNIP stations.

The records for each station include the stable isotope compositions $\delta^2\text{H}$ and $\delta^{18}\text{O}$ and tritium contents with the analytical uncertainties stated by the reporting laboratory. In addition to the isotope data, basic meteorological variables as recorded by the stations such as type and amount of precipitation, surface air temperature and vapour pressure, are also stored in the database.

4. Analysis of GNIP data

4.1. THE METEORIC WATER LINE

The very first analyses of a few meteoric water samples from non-marine environments (Epstein and Mayeda, 1953; Friedman, 1953) indicated that their oxygen and hydrogen isotope values were linearly correlated. Craig (1961) analysed about 400 samples river and lake water, rain, and snow from diverse geographic locations. He found that all of the data, except for those from the lakes, plotted along a line defined by the equation

$$\delta^2\text{H} = 8 \delta^{18}\text{O} + 10 \quad (3)$$

Although Craig did not call it as such in 1961, the above equation became known as the "global meteoric water line (GMWL)" which traced the isotopic compositions of natural waters originating from atmospheric precipitation and not subjected to surface evaporation. The physical basis for this correlation lies in the fractionation of isotopes during evaporation-condensation processes and is discussed in more detail by Gat (this volume).

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Dansgaard (1964), using precipitation data from the early phase of GNIP, found that isotopes in precipitation from northern hemisphere continental stations followed the GWML of Craig, but that significant deviations existed in precipitation data from other continents and from island stations. These deviations from GMWL were attributed to differences in meteorological conditions and processes responsible for precipitation (see also Gat, this volume).

Statistical analyses of GNIP data for the periods 1960-1978 and 1960-1987, respectively, were reported by Yurtsever and Gat (1981) and Rozanski et al. (1993; IAEA, 1992). Using the same methodology described in the above reports, data from 1961-2000 have now been analysed. The arithmetic (unweighted) means of isotope ratios in precipitation from nearly 410 stations are described by the following equation:

$$\delta^2\text{H} = 8.07(\pm 0.02) \delta^{18}\text{O} + 9.9(\pm 0.1) \quad (R^2=0.98) \quad (4)$$

Long term means weighted by the amount of precipitation were calculated considering only the years for which more than 70% of precipitation was analysed for a given isotope and at least one year of observation was available. The correlation between the weighted means is:

$$\delta^2\text{H}_{(\text{weighted})} = 8.14(\pm 0.02) \delta^{18}\text{O} + 10.9(\pm 0.2) \quad (R^2=0.98) \quad (5)$$

The unweighted best-fit line is remarkably close to the line derived by Craig in 1961 from a very small number of observations. This indicates that the basic processes controlling the isotope distributions in precipitation are well understood (see Gat, this volume).

With the relatively large temporal record now available, it is possible to compare decadal datasets for systematic changes in isotope compositions. Thirty-one GNIP stations have reported data throughout the 1961-2000 period. Linear regressions of weighted annual means of δ values from these stations for the periods 1960-1978, 1960-1987, and 1960-2000 are shown in Fig. 4. Although the weighted means for these stations are slightly different than the global relationship discussed above, there is no clear trend of isotope variations from 1961 to 2000. The deuterium excess is about 1 per mil lower for 1960-2000 compared to the 1960-1978 data, but this is most likely due to improvements in measurement techniques and precision rather than changes in climatic conditions.

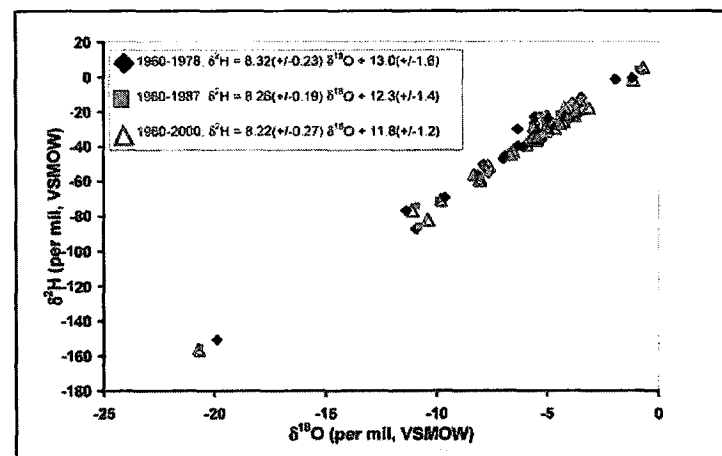


FIG. 4. Weighted annual means of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in precipitation at 31 GNIP stations with a continuous record from 1960 to 2000. The data do not show a discernible pattern of temporal change in isotope values.

4.2. CAUSES OF VARIATIONS IN ISOTOPE COMPOSITIONS

Dansgaard's (1964) comprehensive analysis of global distributions of stable isotopes identified several "effects" on isotope variations resulting from a number of physical and meteorological parameters such as latitude, altitude, distance from the coast, amount of precipitation, and surface air temperature. The reviews that followed (Yurtsever and Gat, 1981; Rozanski et al., 1993; Ingraham, 1998; Gat et al., 2001) substantiated these so-called "effects" which are caused by isotope fractionation associated with phase changes of water in the hydrologic cycle (see also Gat, this volume). Some of these effects are caused by isotope fractionation at the condensation of atmospheric water vapour to precipitation. The air masses lose water as they move along surface temperature gradients from tropical to polar latitudes ("latitudinal effect"), from the sea to inland ("continental effect"), or from lower to higher elevations ("altitude effect").

Figure 5 shows the correlation between weighted means of $\delta^{18}\text{O}$ in precipitation and local air temperature for 325 GNIP stations.

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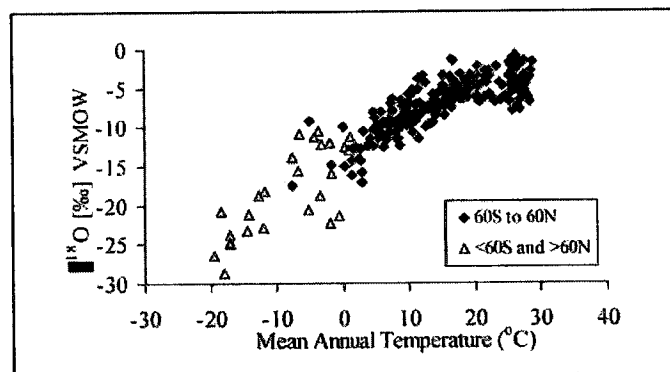


FIG. 5. Correlation between weighted means of $\delta^{18}\text{O}$ in precipitation and local air temperature for 325 GNIP stations.

As described in previous reviews, there is a clear dependence of isotope contents on temperature for stations with annual means of air temperature (t) from about -20°C to $+20^\circ\text{C}$.

For polar stations (-20°C to -2°C), the calculated temperature dependence of $\delta^{18}\text{O}$ is $0.82\text{‰ per }^\circ\text{C}$. Rozanski et al (1993) and Dansgaard (1964), for the same temperature range, reported dependencies of 0.9 and $0.8 \text{‰ per }^\circ\text{C}$. For the temperature range of $0 - 20^\circ\text{C}$, the temperature dependency is lower ($0.53\text{‰ per }^\circ\text{C}$). This compares with earlier estimates of $0.69 \text{‰ per }^\circ\text{C}$ (Dansgaard, 1964) and $0.58 \text{‰ per }^\circ\text{C}$ (Rozanski et al., 1993). It should be noted that Dansgaard's analysis was based on a much smaller dataset compared to the analysis of Rozanski et al. and to this study.

Isotopic compositions of precipitation at stations with a mean annual air temperature higher than about 18°C (mainly tropical stations) do not show a discernible temperature dependence. This lack of temperature dependency in tropical precipitation has been attributed to the more dominant role of the amount of precipitation or the "amount effect" (Dansgaard, 1964; Rozanski et al., 1993; Gat, 1996). Figures 6(a) and 6(b) show the $\delta^{18}\text{O}$ – precipitation amount relationships for all stations, including polar and tropical stations. Isotope–amount relationship is better defined for the polar stations, but overall the relationship is rather poor.

The dependencies of isotope compositions on temperature and amount of precipitation are now considered to be more complex (Aggarwal et al., 2004; Alley and Cuffey, 2001; Fricke and O'Neil, 1999; Hendricks et al., 2000; Hoffman et al., this volume; Jouzel et al., 2000), than were suggested in earlier reviews of GNIP data. These complexities arise from the much greater influence of moisture source and transport patterns on isotope composition compared to the influences of temperature and/or precipitation amount alone.

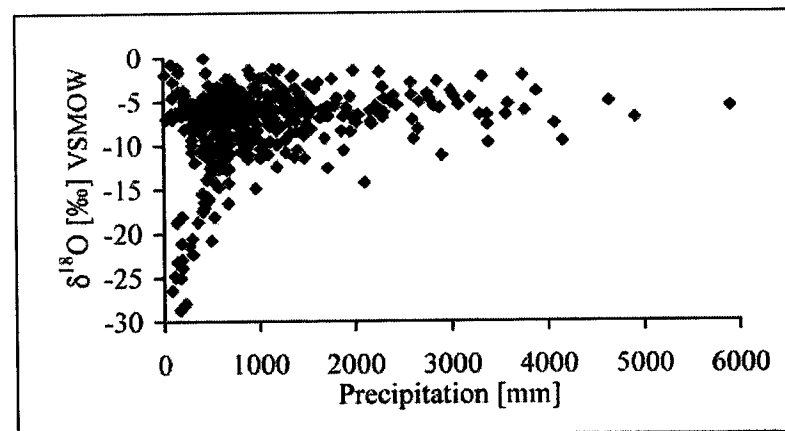


FIG. 6(a). $\delta^{18}\text{O}$ vs. precipitation amount for all GNIP stations.

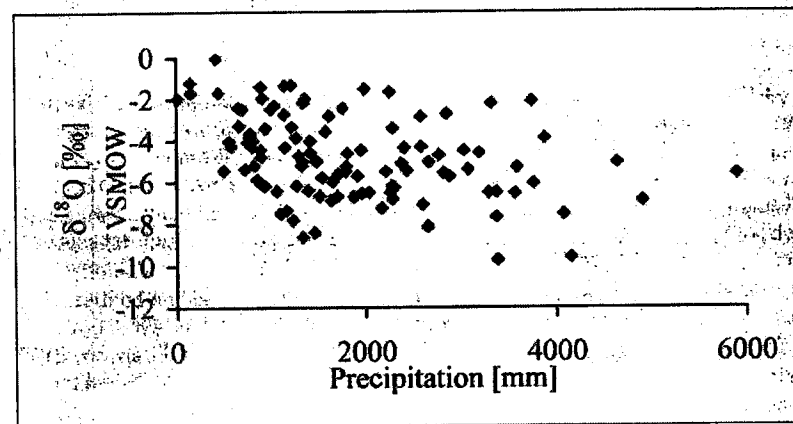


FIG. 6(b). $\delta^{18}\text{O}$ vs. precipitation amount for tropical GNIP stations.

5. Future trends

Isotope data from GNIP have provided a basis for global applications of isotopes in hydrology, palaeoclimatology, and climate change research. While the utility of these data has been wide-ranging, some of the limitations of GNIP also should be recognized. Precipitation samples for GNIP are a monthly composite of all precipitation at a station. Therefore, these data cannot be used to investigate the causes of daily or event-based variations in precipitation processes. The relatively limited geographical density of stations also makes it difficult to use GNIP data for

investigating inter-annual variability in precipitation. An isotope network designed to study precipitation and climate variability at daily scale would include perhaps thousands of stations across the globe, similar to a terrestrial network for climate monitoring on this scale. However, an isotope network for global monitoring of monthly variations of precipitation may only require about one thousand stations. Such a network can be operated with increased cooperation from the scientific community at large and would contribute substantially to improved applications of isotopes in hydrology and hydro-meteorology. The IAEA has made substantial efforts in this direction and it is likely that a strengthened GNIP with a desirable station density and distribution would become available in the next five years. This global network may then be supplemented with additional stations for more detailed local or regional studies of hydrology and climate. Recent efforts of the IAEA to explore the feasibility of global networks of isotopes in river discharge and in terrestrial moisture would complement a global precipitation network. A strengthened GNIP together with isotope data from rivers and terrestrial moisture should spur additional research related to climate modelling, biosphere-hydrosphere interactions, and local or continental scale hydrology.

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**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES**

2020

Bogotá, D.C., 15 de diciembre de 2010

Doctora

Mónica Wolf Wasserman

Representante

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	Folios	001039

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía Nacional	☐	Cap. I	☒
Vía PCT (fase nacional)	☒	Cap. II	☐

No. Sol. Internacional	PCT/RU2005/000045
Fecha de Presentación Internal.	8 de febrero de 2005

Con fundamento en el artículo 46 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

El artículo 46 de la Decisión 486 de la Comisión de la Comunidad Andina establece que:

"La oficina nacional competente podrá requerir el informe de expertos o de organismos científicos o tecnológicos que se consideren idóneos, para que emitan opinión sobre la patentabilidad de la invención. Asimismo, cuando lo estime conveniente, podrá requerir informes de otras oficinas de propiedad industrial."

"De ser necesario, a efectos del examen de patentabilidad y a requerimiento de la oficina nacional competente, el solicitante proporcionará, en un plazo que no excederá de 3 meses, uno o más de los siguientes documentos relativos a una o más de las solicitudes extranjeras referidas total o parcialmente a la misma invención que se examina:

"...b) copia de los resultados de exámenes de novedad o de patentabilidad efectuados respecto a esa solicitud extranjera;...

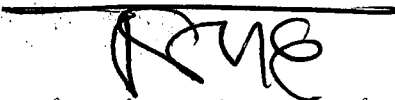
Si el solicitante no presentara los documentos requeridos dentro del plazo señalado en el presente artículo la oficina nacional competente denegará la patente."

Se encontró que la solicitud en estudio, que corresponde con la solicitud internacional PCT/RU2005/000045, corresponde también con la solicitud de Estados Unidos 11/8151888, y que de acuerdo con los exámenes efectuados en fase internacional y en fase nacional en Estados Unidos, se contemplan los siguientes documentos:

- D1 Patent abstracts of Japan. Vol. 2003, No. 12, 5 december 2003. JP2004315487. Abstract
- D2 Patent abstracts of Japan. Vol. 2003, No. 12, 5 december 2003. JP2004075654. Abstract
- D3 Somlyai G et Al. The biological effects of deuterium depleted water, a possible new tool in cancer therapy. Deutsche Zeitschrift fuer onkologie Heidelberg, DE, Vol. 30 No. 4 1998, pages 91-94.
- D4 Bild W et Al: Research concerning the radioprotective and immunostimulating effects of deuterium depleted water. Revue Roumaine de physiologie, editions de l'Academie de la Republique socialiste de la Rouma

Dado que dichos documentos son el resultado de exámenes de patentabilidad efectuados a solicitudes extranjeras que corresponden totalmente con la solicitud colombiana, esta Oficina requiere que el solicitante proporcione en un plazo que no excederá de 3 meses, la copia de los documentos mencionados anteriormente tal y como fueron allegados a la oficina de Estados Unidos en original y con su traducción al idioma inglés. Nos permitimos recordarle que si el solicitante no presenta los documentos requeridos de forma legible y nítida dentro del plazo señalado la oficina nacional competente denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e del capítulo quinto (5), del título primero de la circular única número 10 de 2001.


José Luis Salazar López

Directora de Nuevas Creaciones (E)

Concepto Técnico No. **4161**

Examinador Técnico **202003**

El anterior requerimiento fue notificado por fijación en lista, de fecha 21 ENE 2001