

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 13-263188- -2	FECHA: 2015-06-04 14:38:55
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 27
ACT: 430 REQUERSOLICITA	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
RUBIELA ARIAS DE FAJARDO

Asunto: Radicación: 13-263188- -2
 Trámite: 11
 Evento: 378
 Actuación: 430
 Oficio: 6268
 Folios: 27

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/CR2011/00001				
Fecha de Presentación Internacional	08/04/2011				

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 comunica:

1. Documentos estudiados

Descripción:	Rad. No. 13-263188-00000-0000	07/Noviembre/2013
Reivindicaciones:	Rad. No. 13-263188-00000-0000	07/Noviembre/2013

2. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el

examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso se estudiarán las reivindicaciones 1 a 36 del Rad. No. 13-263128-00000-0000.

Título de la solicitud: “Método para producción de formulaciones inyectables de productos proteicos hemoderivados y productos obtenidos utilizando dicho método”.

El problema planteado en esta solicitud consiste en la necesidad de proporcionar un método más eficiente en la obtención de productos hemoderivados.

Para resolver este problema técnico la solicitud en estudio proporciona un método para la producción de formulaciones inyectables de productos proteicos hemoderivados con carga viral reducida, caracterizado porque comprende los pasos de:

- a. fraccionamiento del material de partida en un sistema de dos fases acuosas mediante la adición de un polímero y al menos una sal;
- b. Adición de fenol al sistema de dos fases como primer paso de inactivación viral;
- c. separación de las fases superior e inferior del sistema de dos fases acuosas;
- d. purificación de los productos de la fase superior del sistema mediante precipitación con un ácido graso;
- e. Purificación de la fase inferior de los productos del sistema mediante termocoagulación;
- f. remoción del precipitado de proteínas desnaturalizadas formado durante los pasos de purificación de la fase superior e inferior del sistema de dos fases;
- ii. aumento de la pureza de los productos obtenidos a partir de la fase superior e inferior mediante cromatografía;
- iii. nanofiltración de los productos obtenidos en el paso anterior para remover partículas virales;
- i. formulación, estabilización y esterilización de los productos obtenidos.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07K 1/14, A61K39/395, A61P7/00 y C07K16/18.

4. Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no es concisa ni se encuentra sustentada porque los términos “polímero, sal, ácido graso, cromatografía y esterilización” son imprecisos y no permiten distinguir la invención del estado de la técnica. Lo anterior considerando que los términos polímero, sal y ácido graso, corresponden a grupos moleculares con características específicas, en el presente caso no existe sustento para todos los compuestos que se encuentran en estos grupos. Por su parte, las expresiones cromatografía y esterilización corresponden a métodos analíticos de separación y de disminución de la carga viral, donde es evidente que la presente solicitud no contiene todos los métodos de separación ni de esterilización. Se sugiere sustituir por términos concretos.

La reivindicación 2 no es clara porque la expresión “Productos proteicos hemoderivados” es imprecisa por lo cual no permite distinguir la invención del estado de la técnica. Se sugiere sustituirlo por un término preciso o un rango que tenga sustento en al descripción.

La reivindicación 3 no es clara porque la expresión “peso molecular entre 1000-6000 Da”, es imprecisa y permite un sinnúmero de polímeros que no se encuentran sustentados. Se sugiere sustituirlo por un rango concreto.

La reivindicación 7 no se encuentra sustentada porque la descripción no describe métodos en donde todas dichas sales hayan sido probadas, se sugiere modificar a una razonable generalización de las sales empleadas en la descripción.

Las reivindicaciones 11 y 20 no son claras porque la expresión “proporción dependiente” es relativo por lo cual no permite distinguir la invención del estado de la técnica. Se sugiere sustituirlo por un término o rango concreto.

La reivindicación 28 no se encuentra sustentada porque no existe sustento en el capítulo descriptivo de todas las modalidades de purificación que se encuentran reclamadas. Se sugiere limitarse a una razonable generalización del tipo de modalidades de purificación que se encuentran descritas.

3. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación internacional: 08 de abril de 2011, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	WO2010062244	“Aqueous two phase extraction augmented precipitation process for purification of therapeutic proteins”	03/Junio/2010
D2	US2007244305	“Process for The Manufacture of Virus Safe Immunoglobulin”	18/Octubre/2007
D3	US4156681	“Process for isolating albumin from blood”	29/Mayo/1979
D4	Transfusion Medicine Reviews, Vol 21, No 2 (April), 2007: pp 101-117	“Modern Plasma Fractionation”	Abril/2007
D5	Toxicon, Vol. 32, No. 3, pp. 351-363, 1994	“Caprylic acid fractionation of hyperimmune horse plasma: description of a simple procedure for antivenom production”	27/Septiembre/1994

El documento D1¹ enseña métodos de producción de productos hemoderivados, que comprende los pasos de separación, inactivación viral, purificación y

1.Url: <https://www.google.com/patents/WO2010062244A1?cl=de>

formulación (Abstract).

El documento D2² revela procesos para la preparación y purificación de inmunoglobulinas a partir de productos plasmáticos, método que comprende los pasos de agregar a la solución ácido caprílico con la finalidad de inactivar proteínas virales, purificar las proteínas con cromatografía de intercambio iónico y procesos de filtración para eliminar partículas virales (Abstract y Párr.[0034]).

El documento D3³ revela métodos de obtención de albuminas a partir de plasma, que comprende los pasos de separación del plasma, adición de un estabilizante, tratamiento con alcoholes, separación por cambios de pH y temperatura, precipitación de los contaminantes y separación que contienen albumina (Reivindicación 1).

El documento D4⁴ divulga métodos de obtención de fracciones de plasma humano con actividad terapéutica, donde éstos comprenden procesos de inactivación de agentes virales, nanofiltración y pasteurización. De la misma forma, el documento D4 divulga fracciones que contienen Ig y IgM, para el tratamiento de enfermedades inmunológicas (Pág. 101-103).

El documento D5⁵ enseña una metodología para el fraccionamiento de plasma que incluye la adición de NaCl y fenol (Abstract).

4. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art18 D486)

Nivel inventivo frente a documento D1 y D5

La reivindicación 1 consiste *“En un método para la producción de formulaciones inyectables de productos proteicos hemoderivados con carga viral reducida, caracterizado porque comprende los pasos de:*

- a. fraccionamiento del material de partida en un sistema de dos fases acuosas mediante la adición de un polímero y al menos una sal;*
- b. Adición de fenol al sistema de dos fases como primer pasó de inactivación viral;*
- c. separación de las fases superior e inferior del sistema de dos fases acuosas;*
- d. purificación de los productos de la fase superior del sistema mediante precipitación con un ácido graso;*
- e. Purificación de la fase interior de los productos del sistema mediante termocoagulación;*
- f. remoción del precipitado de proteínas desnaturalizadas formado durante los pasos de purificación de la fase superior e inferior del sistema de dos fases;*

2.Url:[http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?CC=US&NR=2007244305&KC=&locale=en_EP&FT=E)

CC=US&NR=2007244305&KC=&locale=en_EP&FT=E

3.Url:[http://worldwide.espacenet.com/publicationDetails/originalDocument?](http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=4156681A&KC=A&FT=D&ND=&date=19790529&DB=&locale=en_EP)

CC=US&NR=4156681A&KC=A&FT=D&ND=&date=19790529&DB=&locale=en_EP

4.Url: <http://www.sciencedirect.com/science/article/pii/S0887796306000940>

5 Url: <http://www.sciencedirect.com/science/article/pii/0041010194900876>

- ii. aumento de la pureza de los productos obtenidos a partir de la fase superior e inferior mediante cromatografía;
- iii. nanofiltración de los productos obtenidos en el paso anterior para remover partículas virales;
- i. Formulación, estabilización y esterilización de los productos obtenidos.”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D5
Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados:	Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados (Abstract):	Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados (Abstract):
a. fraccionamiento del material de partida	a. fraccionamiento del material de partida con PEG de 1450 – 6000 Da y fosfato monobásico de sodio	a. Fraccionamiento del material de partida y adición sal(Abstract):
b. Adición de fenol	No menciona	b. adición fenol (Abstract):
c. separación de las fases	c. separación de las fases	c. Separación de fases (Abstract):
d. purificación de los productos y precipitación con un ácido graso	d. Purificación de los productos	d. Purificación de la Ig y adición de ácido caprílico. (Abstract):
e. Purificación de la fase interior de los productos del sistema mediante termocoagulación	e. Purificación por con control de productos	e. Purificación
f. remoción del precipitado	F. eliminación de precipitado	f. eliminación precipitado (Abstract):
Separación	Separación	No menciona
Nanofiltración	Nanofiltración	
formulación, estabilización y esterilización de los productos obtenidos	formulación, estabilización y esterilización de los productos obtenidos	

El documento D1 se considera el estado de la técnica más cercano a la invención porque enseña métodos de producción de productos hemoderivados, que comprende los pasos de:

- a. Fraccionamiento del material de partida con PEG y sales de fosfato monobásico de potasio.
- b. Un proceso de separación de fases por solubilidad.
- c. Purificación de los productos.
- e. eliminación del precipitado
- g. Procesos de formulación. (Reivindicación 1 y Resumen).

Adicionalmente, el documento D1 enseña que valores de pH entre 3.0-9.0, son necesarios para mejorar el proceso de extracción (Reivindicación 19) y métodos de separación cromatográfica (Pág.3 y 10).

La diferencia entre la invención, definida en la reivindicación 1 y el método descrito en el documento D1 consiste en que el método de la solicitud incluye un paso de adición de fenol.

El efecto que logra el incluir un paso que comprende la adición de fenol, en métodos de obtención de productos hemoderivados es favorecer la disminución de la carga viral.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo proporcionar métodos de obtención de productos hemoderivados más eficientes”.

Sin embargo, la inclusión de un paso que comprende la adición de fenol para disminuir la carga viral en métodos de obtención de productos hemoderivados, ya se encontraba divulgada en el documento D5 que se refiere a un método de obtención de productos hemoderivados que comprende la adición de fenol en cantidades adecuadas con la finalidad de disminuir la carga microbiana (FI., Pág., renglón, columna 3, líneas 10-30).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría un paso de adición de fenol del documento D5 en el método de obtención de productos hemoderivados divulgado en el documento D1 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

Conclusión:

La reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones 1-36 no mencionan características adicionales se considera que no tienen nivel inventivo.

Nivel inventivo frente a documento D2 y D5

La reivindicación 1 consiste “*En un método para la producción de formulaciones inyectables de productos proteicos hemoderivados con carga viral reducida, caracterizado porque comprende los pasos de:*

- a. fraccionamiento del material de partida en un sistema de dos fases acuosas mediante la adición de un polímero y al menos una sal;*
- b. Adición de fenol al sistema de dos fases como primer pasó de inactivación viral;*
- c. separación de las fases superior e inferior del sistema de dos fases acuosas;*
- d. purificación de los productos de la fase superior del sistema mediante precipitación con un ácido graso;*
- e. Purificación de la fase interior de los productos del sistema mediante termocoagulación;*
- f. remoción del precipitado de proteínas desnaturalizadas formado durante los pasos de purificación de la fase superior e inferior del sistema de dos fases;*
- ii. aumento de la pureza de los productos obtenidos a partir de la fase superior e inferior mediante cromatografía;*

- iii. nanofiltración de los productos obtenidos en el paso anterior para remover partículas virales;
i. Formulación, estabilización y esterilización de los productos obtenidos.”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D2	D5
Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados:	Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados (Abstract):	Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados (Abstract):
a. fraccionamiento del material de partida	a. fraccionamiento del material de partida con PEG (Párr. [0019])	a. Fraccionamiento del material de partida y adición sal
b. Adición de fenol	No menciona	b. adición fenol
c. separación de las fases	c. separación de las fases	c. Separación de fases
d. purificación de los productos y precipitación con un ácido graso	d. purificación de los productos y precipitación con ácido caprílico	d. Purificación de la Ig y adición de ácido caprílico.
e. Purificación de la fase interior de los productos del sistema mediante termocoagulación	e. Purificación productos (Párr. [0047])	e. Purificación
f. remoción del precipitado	F. eliminación de precipitado	f. eliminación precipitado
Separación	Separación	No menciona
Nanofiltración	Nanofiltración (Párr. [0047])	
formulación, estabilización y esterilización de los productos obtenidos	formulación, estabilización y esterilización de los productos obtenidos	

El documento D2 se considera el estado de la técnica más cercano a la invención porque enseña métodos para la preparación y purificación de inmunoglobulinas a partir de productos plasmáticos, donde este método comprende los pasos de adición de ácido caprílico con la finalidad de inactivar proteínas virales, purificar las proteínas con cromatografía de intercambio iónico y procesos de filtración para eliminar partículas virales (Abstract y Párr.[0034]).

La diferencia entre la invención, definida en la reivindicación 1 y el método descrito en el documento D2 consiste en que el método de la solicitud incluye un paso de adición de fenol.

El efecto que logra el incluir un paso que comprende la adición de fenol, en métodos de obtención de productos hemoderivados es favorecer la disminución de la carga viral.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo proporcionar métodos de obtención de productos hemoderivados más eficientes”.

Sin embargo, la inclusión de un paso que comprende la adición de fenol para disminuir la carga viral en métodos de obtención de productos hemoderivados, ya se encontraba divulgada en el documento D5 que se refiere a un método de obtención de productos hemoderivados que comprende la adición de fenol con la finalidad de disminuir la carga (Abstract).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría un paso de adición de fenol del documento D5 en el método de obtención de productos hemoderivados divulgado en el documento D2 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

Conclusión:
La reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones 1-36 no mencionan características adicionales se considera que no tienen nivel inventivo.

Nivel inventivo frente a documento D3 y D5

La reivindicación 1 consiste *“En un método para la producción de formulaciones inyectables de productos proteicos hemoderivados con carga viral reducida, caracterizado porque comprende los pasos de:*

- a. fraccionamiento del material de partida en un sistema de dos fases acuosas mediante la adición de un polímero y al menos una sal;*
- b. Adición de fenol al sistema de dos fases como primer pasó de inactivación viral;*
- c. separación de las fases superior e inferior del sistema de dos fases acuosas;*
- d. purificación de los productos de la fase superior del sistema mediante precipitación con un ácido graso;*
- e. Purificación de la fase interior de los productos del sistema mediante termocoagulación;*
- f. remoción del precipitado de proteínas desnaturalizadas formado durante los pasos de purificación de la fase superior e inferior del sistema de dos fases;*
- ii. aumento de la pureza de los productos obtenidos a partir de la fase superior e inferior mediante cromatografía;*
- iii. nanofiltración de los productos obtenidos en el paso anterior para remover partículas virales;*
- i. Formulación, estabilización y esterilización de los productos obtenidos.”*

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D3	D5
Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados:	Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados (Ej.9).	Un método para la producción de formulaciones inyectables de productos proteicos

		hemoderivados (Abstract):
a. fraccionamiento del material de partida	a. fraccionamiento del material de partida con un estabilizador albumina (Ej.9).	a. Fraccionamiento del material de partida y adición sal
b. Adición de fenol	b) adición de alcoholes alifáticos (Ej.9).	b. adición fenol
c. separación de las fases	c. separación de las fases (Ej.9)	c. Separación de fases
d. purificación de los productos y precipitación con un ácido graso	d. purificación de los productos y precipitación con ácido caprílico (Ej.1-9).	d. Purificación de la Ig y adición de ácido caprílico.
e. Purificación de la fase interior de los productos del sistema mediante termocoagulación	e. Purificación productos (Ej.1-9).	e. Purificación
f. remoción del precipitado	F. eliminación de precipitado (Ej.1-9).	f. eliminación precipitado
Separación	Separación	No menciona
Nanofiltración	Nanofiltración	
formulación, estabilización y esterilización de los productos obtenidos	formulación, estabilización y esterilización de los productos obtenidos (Ej. 1)	

El documento D3 se considera el estado de la técnica más cercano a la invención porque revela métodos de obtención de albuminas a partir de plasma, que comprende los pasos de separación del plasma, adición de un estabilizante, tratamiento con alcoholes, separación por cambios de pH y temperatura, precipitación de los contaminantes y separación que contienen albumina (Reivindicación 1).

La diferencia entre la invención, definida en la reivindicación 1 y el método descrito en el documento D3 consiste en que el método de la solicitud incluye un paso de adición de fenol.

El efecto que logra el incluir un paso que comprende la adición de fenol, en métodos de obtención de productos hemoderivados es favorecer la disminución de la carga viral.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo proporcionar métodos de obtención de productos hemoderivados más eficientes”.

Sin embargo, la inclusión de un paso que comprende la adición de fenol para disminuir la carga viral en métodos de obtención de productos hemoderivados, ya se encontraba divulgada en el documento D5 que se refiere a un método de obtención de productos hemoderivados que comprende la adición de fenol con la finalidad de disminuir la carga (Abstract).

En consecuencia, la persona del oficio normalmente versada en la materia,

incluiría un paso de adición de fenol del documento D5 en el método de obtención de productos hemoderivados divulgado en el documento D3 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

Conclusión:
La reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones 1-36 no mencionan características adicionales se considera que no tienen nivel inventivo.

Nivel inventivo frente a documento D4 y D5

La reivindicación 1 consiste “En un método para la producción de formulaciones inyectables de productos proteicos hemoderivados con carga viral reducida, caracterizado porque comprende los pasos de:

- a. fraccionamiento del material de partida en un sistema de dos fases acuosas mediante la adición de un polímero y al menos una sal;
- b. Adición de fenol al sistema de dos fases como primer pasó de inactivación viral;
- c. separación de las fases superior e inferior del sistema de dos fases acuosas;
- d. purificación de los productos de la fase superior del sistema mediante precipitación con un ácido graso;
- e. Purificación de la fase interior de los productos del sistema mediante termocoagulación;
- f. remoción del precipitado de proteínas desnaturalizadas formado durante los pasos de purificación de la fase superior e inferior del sistema de dos fases;
- ii. aumento de la pureza de los productos obtenidos a partir de la fase superior e inferior mediante cromatografía;
- iii. nanofiltración de los productos obtenidos en el paso anterior para remover partículas virales;
- i. Formulación, estabilización y esterilización de los productos obtenidos.”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D4	D5
Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados:	Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados (Tabla3).	Un método para la producción de formulaciones inyectables de productos proteicos hemoderivados (Abstract):
a. fraccionamiento del material de partida	a. fraccionamiento del material de partida (Tabla3).	a. Fraccionamiento del material de partida y adición sal
b. Adición de fenol	b) adición de alcoholes alifáticos (Tabla3).	b. adición fenol
c. separación de las fases	c. separación de las fases (Ej.9)	c. Separación de fases
d. purificación de los	d. purificación de los	d. Purificación de la Ig y

productos y precipitación con un ácido graso	productos y precipitación con ácido caprílico (Tabla3).	adición de ácido caprílico.
e. Purificación de la fase interior de los productos del sistema mediante termocoagulación	e. Purificación productos (Tabla3).	e. Purificación
f. remoción del precipitado	F. eliminación de precipitado (Tabla3).	f. eliminación precipitado
Separación	Separación	No menciona
Nanofiltración	Nanofiltración	
formulación, estabilización y esterilización de los productos obtenidos	formulación, estabilización y esterilización de los productos obtenidos (Ej. 1)	

El documento D4 se considera el estado de la técnica más cercano a la invención porque divulga métodos de obtención de fracciones de plasma humano con actividad terapéutica, donde éstos comprenden procesos de inactivación de agentes virales, nanofiltración y pasteurización. De la misma forma, el documento D4 divulga fracciones que contienen Ig y IgM, para el tratamiento de enfermedades inmunológicas (Pág. 101-103).

La diferencia entre la invención, definida en la reivindicación 1 y el método descrito en el documento D4 consiste en que el método de la solicitud incluye un paso de adición de fenol.

El efecto que logra el incluir un paso que comprende la adición de fenol, en métodos de obtención de productos hemoderivados es favorecer la disminución de la carga viral.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo proporcionar métodos de obtención de productos hemoderivados más eficientes”.

Sin embargo, la inclusión de un paso que comprende la adición de fenol para disminuir la carga viral en métodos de obtención de productos hemoderivados, ya se encontraba divulgada en el documento D5 que se refiere a un método de obtención de productos hemoderivados que comprende la adición de fenol con la finalidad de disminuir la carga (Abstract).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría un paso de adición de fenol del documento D5 en el método de obtención de productos hemoderivados divulgado en el documento D4 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

Conclusión:

La reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones 2-36 no mencionan características adicionales se

considera que no tienen nivel inventivo.

5. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO2010062244	1-36	Nivel inventivo
D2	US2007244305	1-36	Nivel inventivo
D3	US4156681	1-36	Nivel inventivo
D4	Transfusion Medicine Reviews, Vol 21, No 2 (April), 2007: pp 101-117	1-36	Nivel inventivo
D5	Toxicon, Vol. 32, No. 3, pp. 351-363, 1994	1-36	Nivel inventivo

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 (60) días contados a partir del día siguiente 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.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

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 No. 10 de 2001.



JESUS FERNEL GARCÍA GARCÍA
Director de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-06-09

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Modern Plasma Fractionation

Thierry Burnouf

Protein products fractionated from human plasma are an essential class of therapeutics used, often as the only available option, in the prevention, management, and treatment of life-threatening conditions resulting from trauma, congenital deficiencies, immunologic disorders, or infections. Modern plasma product production technology remains largely based on the ethanol fractionation process, but much has evolved in the last few years to improve product purity, to enhance the recovery of immunoglobulin G, and to isolate new plasma proteins, such as α 1-protease inhibitor, von Willebrand factor, and protein C. Because of the human origin of the starting material and the pooling of 10 000 to 50 000 donations required for industrial processing, the major risk associated to plasma products is the transmission of blood-borne infectious agents. A complete set of measures—and, most particularly, the use of dedicated viral inactivation and removal treatments—has been implemented throughout the production chain

of fractionated plasma products over the last 20 years to ensure optimal safety, in particular, and not exclusively, against HIV, hepatitis B virus, and hepatitis C virus. In this review, we summarize the practices of the modern plasma fractionation industry from the collection of the raw plasma material to the industrial manufacture of fractionated products. We describe the quality requirements of plasma for fractionation and the various treatments applied for the inactivation and removal of blood-borne infectious agents and provide examples of methods used for the purification of the various classes of plasma protein therapies. We also highlight aspects of the good manufacturing practices and the regulatory environment that govern the whole chain of production. In a regulated and professional environment, fractionated plasma products manufactured by modern processes are certainly among the lowest-risk therapeutic biological products in use today.

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COLLECTED HUMAN PLASMA may be used as a therapeutic product (known as “clinical plasma” or “fresh frozen plasma”) or as source material for the production of pharmaceutical fractionated products (also called “plasma products” or “plasma derivatives”). This complex biologic material contains hundreds of proteins covering a myriad of physiological functions. Many components still have undiscovered roles. The most abundant proteins, albumin and immunoglobulin (Ig) G, are present at about 35 and 10 g/L, respectively, representing about 80% of all plasma proteins. Less abundant proteins include the protease inhibitors, like α 1-antitrypsin (AAT) (1.5 g/L) and antithrombin (AT) (300 mg/L), and the coagulation factors such as factor VIII (FVIII) (a few ng/L), which exhibit potent physiologic activity. Currently, about 20 different plasma protein therapeutics are used for treating life-threatening diseases or injuries associated to bleeding and thrombotic disorders, immunological diseases, infectious conditions, as well as tissue degenerating diseases, thus addressing the clinical needs of countless patients. An updated list of the major therapeutic applications of plasma protein products can be found elsewhere.¹

This industrial process used to isolate therapeutic plasma proteins is known as “fractionation.” Over 23 to 28 million liters of human plasma are fractionated each year in the world, in batches of several

thousand liters, in about 70 factories. Modern plasma fractionation combines manufacturing steps to isolate, in a sequential and integrated manner, the crude fractions that are further purified into individual therapeutic products. Validated dedicated steps inactivate and/or remove infectious agents potentially present in the starting plasma pool. This sophisticated industrial process is performed under highly hygienic conditions in licensed facilities (plasma fractionation plants) that are operated in compliance with good manufacturing practices and following quality assurance principles.

Over the years, plasma fractionation has evolved from a medical service activity mostly oriented toward the needs of local communities into a global manufacturing industry conforming to high regulatory standards. These strict requirements start from the collection of plasma for fractionation and include product manufacture and distribution steps. In this article, we review the most current practices encompassing the collection of plasma for fractionation, the core industrial plasma fractionation

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process, and the purification and pathogen reduction technologies of individual plasma products.

PRODUCTION OF PLASMA FOR FRACTIONATION

The practices used for the collection of plasma for fractionation have direct influence on the safety profile of protein products since individual donations contribute to large plasma pools used to manufacture therapeutic preparations intended for hundreds or even thousands of patients. It is therefore logical that the production of plasma is regarded as an integral part of the manufacture of modern fractionated products. Collection requirements of plasma for fractionation may differ from those relevant to fresh frozen plasma. In a regulated environment, plasma for fractionation is collected by licensed/registered blood establishments (blood centers and apheresis collection centers) that are inspected by the relevant National Regulatory Authorities (NRAs). Compelled by the same safety concerns, the plasma fractionators conduct audits to verify that the contractual plasma collection and quality and safety measures, agreed upon with the plasma supplier, are met. Areas of specific relevance include (a) procedures for donor screening and donation testing; (b) labeling, documentation, and traceability requirements; and (c) the handling of blood and plasma. Such information is part of the marketing license of plasma products and, in Europe, is assembled into a document called the "Plasma Master File." Various requirements for the collection of plasma for fractionation have been described in various guides, eg, from the Pharmaceutical Inspection Convention and Pharmaceutical Inspection Cooperation Scheme (jointly referred to as PIC/S), US Food and Drug Administration (FDA) and in recent World Health Organization Recommendations.¹ They are summarized below.

Donors Screening

Candidate donors are provided with educational materials and undergo a medical interview to establish the absence of risks or signs of infections and to prove compliance for a donation of plasma for fractionation (Table 1). Potential donors presenting a health hazard are asked to exclude themselves. Medical information of donors is acquired and archived. Continuous epidemiologic surveillance of the donor population is being required in some jurisdictions.² It helps to establish

the background level (prevalence and incidence) and trends of known infectious markers (eg, HIV 1 and 2 antibodies, hepatitis C virus [HCV] antibodies, and hepatitis B surface antigen [HBsAg]) in the population. This is also of interest for the early detection of emerging diseases, allowing early implementation of counter measures (such as more stringent donor screening processes or requirements for additional testing procedures).

Donors eligible to donate plasma for fractionation are individuals who meet donation criteria (such as age and donation frequency), do not present risk factors of blood-borne infectious agents, and comply with requirements defined by the plasma fractionator and the NRAs of the country of plasma collection and of use of the products. In most situations, eligibility of whole blood donors and apheresis donors overlap, apart from donation frequency which is higher for plasmapheresis donors. Eligibility criteria take into account scientific information about the risks of transmission of infectious agents by pooled plasma products (which may differ from those by blood components). Special criteria may exist for the collection of hyperimmune plasma (used to make hyperimmune IgG preparations), such as procedures for donors' immunization and minimal antibody titer.¹

Collection Methods

Currently, about 35% of the plasma fractionated in the world is obtained by centrifugation of whole blood ("recovered" plasma), and 65% is obtained by apheresis. Blood/plasma collection, processing, and storage may affect plasma quality, as well as having an impact on the recovery of the most labile proteins such as FVIII. In particular, risks of activation of the coagulation, complement, and fibrinolytic systems, which may lead to generation of plasma proteases, should be avoided. To better preserve the integrity of recovered plasma and limit risks of activation of the coagulation cascade and of cellular components, (a) good mixing of the blood with the anticoagulant solution (a sodium citrate based solution¹) should be ensured from the initiation till the end of the collection process; (b) the duration of the collection should not exceed 15 minutes; and (c) temperature variations of the blood should be avoided. A few hours after donation, whole blood is subjected to a centrifugation that separates the cellular elements (most specifically red cells) from plasma. The mean

Table 1. Most Relevant Measures Taken to Prevent the Transmission of Plasma-Borne Infectious Agents by Fractionated Plasma Products

Infectious agent	Blood establishment		Plasma fractionator				
	Donor screening (exclusion criteria)	Individual serologic testing	Mini-pool NAT*	Manufacturing pool testing†	Viral inactivation treatments	Removal by purification steps‡	Removal by nanofiltration§
HIV I and II	Questionnaire	Anti-HIV 1 and 2	Yes	Anti-HIV 1 and 2; HIV NAT	+	(+)	+
HBV	Questionnaire	HBsAg	Yes	HBsAg; HBV NAT	+	(+)	+
HCV	Questionnaire	Anti-HCV	Yes	Anti-HCV; HCV NAT	+	(+)	+
Hepatitis delta virus	(questionnaire)	ND	ND	ND	+	(+)	+
HAV	ND	ND	Yes	HAV NAT	±	(+)	+
Hepatitis E virus	ND	ND	ND	ND	+	(+)	+
Hepatitis G virus	ND	ND	ND	ND	+	(+)	+
TT virus	ND	ND	ND	ND	+	(+)	+
B19	ND	ND	Yes	B19 NAT	±	(+)	+
WNV	ND	ND	ND	ND	+	(+)	+
vCJD	Questionnaire	ND	Not relevant	ND	Not relevant	(+)	(+)

+ Indicates major contribution to safety; ±, contribution depends on type of treatment; ND, not done (or test not available).

*Performed by most fractionators.

†Mandatory in Europe for HCV.

‡May contribute to viral clearance but does not necessarily result in robust and consistent removal.

§For small viruses, robust removal is achieved by narrow pore size membranes (≤20 nm).

||Expected contribution based on experimental studies using spiked TSE agents, in the absence of information of the biological nature of the TSE-human plasma associated agent.

plasma volume obtained from one whole blood donation is about 220 mL but varies depending upon the volume of collected whole blood (most often 400-450 mL) and donor's hematocrit. Apheresis plasma (also called "source plasma") is collected from donors through a process where blood is removed from the donor, anticoagulated (generally with a 4% sodium citrate solution),¹ and immediately separated by physical means (centrifugation or filtration, or a combination of both) into components.³ At minimum, the red cells are returned to the donor while plasma is retained and collected in a container (bag or plastic bottle). The duration of a typical plasmapheresis procedure depends on the number of cycles (and, hence, the volume of plasma collected) and lasts generally from 35 to 70 minutes. Apheresis plasma volume may range from 450 to 880 mL, depending upon the country's regulations and collection protocol. Apheresis plasma can also be prepared as a by-product of plateletpheresis ("concurrent plasma"),

a procedure used primarily for the collection of platelets.

Both recovered and apheresis plasmas are suitable for the manufacture of the whole range of fractionated plasma products. The mean content in coagulation factors, more particularly FVIII, is lower in recovered than in apheresis plasma because of (a) longer processing time before freezing (whole blood must be further processed to separate cellular components and plasma), (b) higher ratio of anticoagulant and, possibly, (c) the higher level of cellular contamination that may release proteolytic enzymes affecting the stability of coagulation factors. Apheresis plasma contains less IgG when collected from frequent donors. Protein content and quality of fractionated proteins is apparently not affected by the apheresis system used, although residual cell content differ based upon the type and configuration of the cell separation device.⁴ Plasma from membrane apheresis procedures, as does recovered plasma prepared from whole blood leukoreduced on positively charged filters, may

contain more activated complement component 3 and 5 (C3a, C5a) anaphylatoxins,⁵⁻⁷ but the impact on the quality or yield of fractionated products is unknown.

Testing of Pathogenic Agents

Various infectious agents have been identified as potential contaminants of human blood.⁸ Bacteria, parasites, and intracellular viruses are not transmitted by plasma products because they are destroyed by freeze-thaw steps or removed by the 0.2- to 1- μ m filtration steps used during the processing of fractionated products. Pathogenic plasma-borne viruses include HIV, HCV, hepatitis B virus (HBV), West Nile virus (WNV), hepatitis A virus (HAV) and parvovirus B19 (B19). The various complementary safety nets in place during the production chain of fractionated products, from donor selection to industrial product extraction, to optimize safety against these agents are summarized in Table 1. The importance of viral testing on the safety of plasma products has been reviewed.^{1,9,10} The extent of viral testing of plasma for fractionation takes into account the ability of validated fractionation processes to eliminate viral risks.¹¹ Some testing is performed by blood establishments, other by plasma fractionators (Table 1). Individual plasma donations must be negative for anti-HIV 1 and 2, anti-HCV, and HBsAg. Genomic assays of plasma minipools for nonenveloped HAV and B19 may be performed.^{11,12} Relevance of testing for the absence of HIV P24Ag or WNV nucleic acid testing (NAT), which may be justified for the safety of non-virally inactivated blood components, is arguable for plasma for fractionation subjected to robust viral reduction steps of enveloped viruses. The industrial manufacturing pool (usually the cryo-poor plasma that is the first homogeneous pooled plasma fraction) is also tested to confirm the absence of serologic and/or genomic viral markers of HIV, HBV, HCV, HAV, and B19. In spite of the most rigorous donor screening and donation testing, infectious viruses may still be present in plasma fractionation pools. Therefore, the viral inactivation-removal steps that have been deliberately introduced during plasma products manufacture—and that are described below—play a most critical role in ensuring safety. Altogether, these overlapping tests should ensure that the viral load of the manufacturing should be both minimal

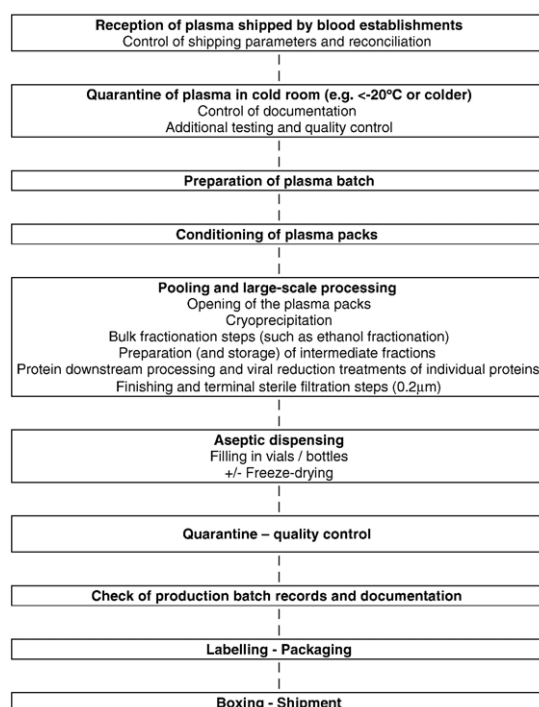


Fig 1. Plasma fractionation flow-chart.

and significantly below the viral reduction capacity of the manufacturing processes used.

Processing, Freezing, Storage, and Transportation of Plasma

Preserving FVIII during blood/plasma collection and preparation is important for most fractionators preparing coagulation factor concentrates. Apheresis plasma can generally be frozen quickly, ensuring optimal FVIII preservation. By contrast, whole blood has to be centrifuged to separate the various components. When blood is cooled to 4°C after collection, plasma should be separated and frozen within 6 to 8 hours to preserve FVIII, but when cooled rapidly at constant 20°C using devices like butanediol plates, coagulation factors are stable for up to 18 to 24 hours. Plasma frozen within 72 hours is suitable for IgG and albumin production.¹ After separation from cellular elements, plasma for fractionation should be frozen rapidly below -20°C, -25°C, or -30°C, depending upon local regulations.¹³ Plasma used to manufacture only albumin and IgG may be frozen below -20 °C within 72 hours of collection.¹³ In the US Code of Federal Regulations, apheresis

Table 2. Typical Plasma Protein Downstream Purification Methods

Method	Description	Separation principle	Application
Cryoprecipitation	Thawing of whole plasma; at +1°C to +4°C	Differential solubility at cold positive temperature	Precipitation of FVIII, VWF, and fibrinogen
Ethanol precipitation	Successive precipitation steps of cryo-poor plasma by Ethanol (10%-40%), under precise conditions of pH (ca 7.4-4.5), temperature (−3 to −6°C), protein concentration, and ionic strength Removal of precipitates by centrifugation or depth-filtration	Differential solubility in ethanol at cold negative temperature	Precipitation of fibrinogen, IgG, albumin, AAT, etc
Ion exchange chromatography	Binding of proteins on a solid support usually packed in a column. Can also be done as a batch process, eg, DEAE, QAE, CM...	Electric charge binding. Elution by increasing salt content or changing pH	Most coagulation factors, protease inhibitors, and anticoagulants
Affinity chromatography	Binding of proteins on a solid support most usually packed in a column; ligands include heparin, metals, and gelatin	Specific affinity ligand proteins. Elution usually by increasing salt content	AT, VWF, FIX, etc
Immunoaffinity	Binding of proteins on a solid support packed in a column. Ligands include murine monoclonal antibodies	Specific affinity antibodies-proteins. Elution usually by increasing salt content	FVIII, FIX, protein C
Size-exclusion chromatography	Injection of proteins on a solid support packed in a column	Separation based on differential molecular mass	AAT, FVIII
Ultrafiltration	Selective fractionation process on membranes of defined pore size that concentrates protein and removes low-molecular-weight solutes and salts	Separation based on differential molecular mass	All products
Microfiltration	Low-pressure cross-flow membrane process for separating colloidal and suspended particles in the range of 0.2 to 10 μm		All products

plasma should be stored at -20°C or colder immediately after collection. Rapid plasma freezing, to ensure rapid ice front velocity and core temperature of -20°C , preserves FVIII and appears more important than the actual freezing temperature itself.¹⁴ Plasma for fractionation is stored at less than -20°C , or colder, typically for several months or more. Storage temperature should be as constant as possible, including the transportation to the fractionation facilities. Cross-continent or

intercontinent shipment of plasma for fractionation is frequent.

INDUSTRIAL PROCESSING OF PLASMA

Production Flow Chart

Production steps taking place at fractionation plants are summarized in Figure 1, and typical plasma protein downstream methods are presented in Table 2. Physical compliance with shipping requirements is verified at delivery of the plasma

(packaging; labeling; shipping temperature as recorded, eg, on data loggers; availability of samples for additional testing, etc). Plasma is immediately quarantined in a walk-in freezer. Documentation is verified (such as signed certificate of origin and of control of the plasma; collection date, shipping container number, virology and immunohematology screening data; test kits used and batch number) and reconciled with actual delivery. Additional testing, such as mini-pool NAT (eg, HCV, HIV, HBV, HAV, and/or B19) and/or statistical determination of FVIII and protein content by sacrificial sampling of individual plasma donations may be done. Only individual plasma donations that comply with quality and testing requirements are used for fractionation. Before thawing, donations may be "conditioned" at controlled temperature for a few hours to facilitate opening of the plasma packs. Various methods, compatible with hygienic processing to limit bioburden, have been developed for pack opening. Frozen plasmas are expelled from the plastic containers and pooled for cryoprecipitation and further manufacturing steps, as described below. Intermediate fractions generated during production may be stored for subsequent pooling and processing. Purified sterile-filtered products are aseptically dispensed into final containers (glass vials or bottles). Albumin bottles undergo terminal pasteurization. Many products, but albumin and some IgG preparations, are freeze-dried, typically for a duration of 3 to 6 days, depending upon physico-chemical characteristics and filled volumes. Batches are quarantined while quality controls and checks of production files take place. Batches meeting specifications are labeled and packaged and subsequently boxed and shipped for distribution. In a few countries, product batches may be released by regulatory authorities. The production cycle of fractionated products takes a few weeks to several months.

Core Fractionation Technology

Current core fractionation technology largely relies on a backbone process encompassing cryoprecipitation and cold ethanol precipitation steps, as developed in the 1940s by Cohn et al¹⁵ in the United States, or modified by Kistler and Nitschman¹⁶ in Europe. This process involves successive processing steps at defined ethanol concentrations, associated with shifts in pH,

temperature, and osmolality that result in selective precipitation of proteins, most notably IgG and albumin. Precipitates are separated by centrifugation or filtration. In the last few years, the complexity of the fractionation process has increased by (a) the introduction of chromatography to isolate new proteins from existing fractions such as cryoprecipitate, cryo-poor plasma, and Cohn fractions; (b) the integration of chromatography to the ethanol fractionation process to increase IgG recovery; and (c) the implementation of dedicated viral inactivation or removal steps. Chromatography was introduced in the 1960s; however, its application developed mostly in the mid/late 1980s. Anion-exchange chromatography and affinity chromatography are frequently used to capture proteins at physiological pH and ionic strength, therefore best preserving functional activity.^{17,18} Immobilized heparin and monoclonal antibodies are common affinity chromatography ligands. Chromatography is used for 4 specific goals: (a) improvement of products purity, (b) extraction of trace labile proteins, (c) optimization of protein recovery, and (d) removal of viral inactivation agents.

Figure 2 illustrates a typical industrial fractionation scheme of standard plasma. Plasma packs (typically for a batch of 2000–4000 L) are opened under hygienic conditions, and plasma is expelled from the containers and thawed at 1°C to 4°C. Cryoprecipitate is isolated using refrigerated continuous centrifuges, recovered from the centrifugation bowls and frozen at –30°C or colder for storage until further pooling and processing. The cryo-poor plasma is immediately processed for primary chromatographic capture of labile coagulation factors (such as the factor IX [FIX] complex and its components) and protease inhibitors (such as AT and C1 esterase inhibitor [C1-inh]). The prepurified intermediates may be stored frozen until further processing. The coagulation factors/anticoagulant-depleted plasma undergoes sequential ethanol precipitation steps. This leads to successive precipitations of fibrinogen, IgG and albumin fractions, and intermediates for extraction of other therapeutic proteins, such as AAT (fraction IV-1), or IgM (fraction III). Depth filtration is preferred to centrifugation to separate precipitates and improve protein recovery. The fractionation of hyperimmune plasma (eg, anti-rhesus) is usually performed on small plasma batch sizes, increas-

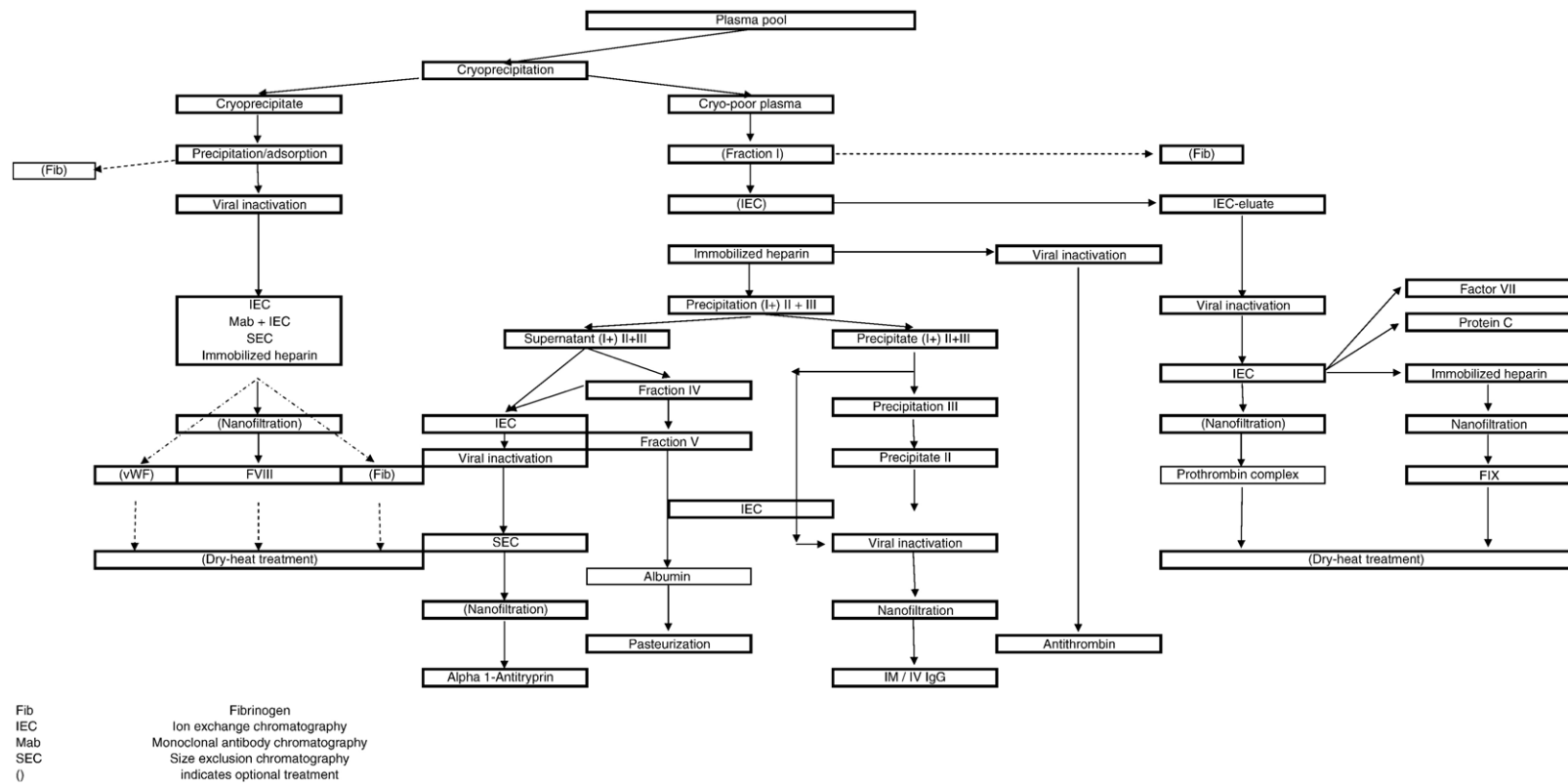


Fig 2. Typical fractionation scheme.

ingly using full chromatographic processes to optimize the recovery of IgG.

Core Viral Reduction Methods

No documented transmission of HIV, HBV, or HCV by products subjected to dedicated viral inactivation treatments has been recorded since the end of the 1980s.¹⁹ Viral reduction treatments include inactivation steps (where viruses are “killed”) and removal steps (where viruses and proteins partition into distinct fractions). Use of one, or preferably two, distinct dedicated viral reduction treatments is the current “gold standard” for all plasma products. The first treatment is performed primarily to inactivate the most pathogenic viruses (HIV, HBV, and HCV), whereas the second reduction step targets nonenveloped viruses but also contributes to added safety against all agents. Most viral reduction treatments are integrated with the protein fractionation process (“in-process” treatments), but some currently based on heat inactivation procedures are applied on products filled in their final container (terminal treatment). Fractionators are required by regulatory authorities to conduct down-scale experimental validation studies using relevant model viruses to establish the efficacy and robustness of viral reduction procedures. The robustness of the viral reduction procedures in place is exemplified by the absence of transmission of WNV, an emerging virus.²⁰ Similarly, the lipid-enveloped severe acute respiratory syndrome coronavirus has been shown to be inactivated by core viral inactivation treatments of plasma products.^{21,22} It is also likely, but not proven by validation experiments yet, that avian flu and simian foamy viruses, which also have a lipid envelope, would be inactivated by current processes in place if present in plasma. Details on viral validation procedures can be found elsewhere.^{23,24} The major characteristics of current viral reduction treatments are summarized in Table 3^{9,23} and discussed briefly here.

In-process viral inactivation treatments. The solvent-detergent (SD) treatment, developed in the mid 1980s,²⁵ remains the most frequent core viral inactivation procedure of plasma products. Protein solutions are incubated for 4 to 6 hours at 24°C to 37°C in the presence of 0.3% to 1% Tri-*n*-butyl phosphate (TnBP) and 1% Tween-80 or Triton X-100. Typically, lipid enveloped viruses are inacti-

vated in a matter of minutes, and the functional activity of even the most labile plasma proteins—with the possible exception of some serine protease inhibitors—is well preserved, but nonenveloped viruses (less pathogenic in most individuals) are not inactivated. The SD agents are removed down to a level of a few parts per million usually by chromatographic adsorption or specific precipitation of proteins, or selective adsorption on hydrophobic chromatographic support.

Pasteurization, another common viral inactivation procedure, is a heat treatment of protein solutions for 10 hours at 60°C, a treatment that denatures viral proteins and inhibits virus replication.²⁶ Pasteurization can inactivate both enveloped and nonenveloped viruses, but stabilizers, needed to limit loss of protein functionality, may decrease the rate and extent of viral inactivation.²⁷ Stabilizers may be removed by ultrafiltration, protein precipitation, or chromatography. Vapor heat has also been used by one company; extent of virus inactivation is influenced by the temperature, duration, and pressure during treatment. Risk of neoantigen formation, which can enhance protein immunogenicity, should be considered when using heat-based inactivation processes.

Low pH incubation, usually at pH 4, at 30°C to 37°C for more than 20 hours, was introduced in the early 1980s to allow the intravenous infusion of IgG. This form of treatment was subsequently found to inactivate most lipid-enveloped viruses. Caprylic (octanoic) acid precipitation/incubation at pH below 6 is a recently introduced treatment of human IgG that can inactivate lipid-enveloped viruses.^{28,29}

Terminal viral inactivation treatments. In modern plasma fractionation, heat treatment of lyophilized products (dry heat) is used, due to limitations, mostly as a secondary viral inactivation step rather than the core inactivation treatment. The treatment is applied to some coagulation factor concentrates. Performed at 80°C for 72 hours or at 100°C for 30 minutes, generally in the presence of protein stabilizers, it provides added safety against HAV and other heat-sensitive viruses but may not be sufficient to exclude B19 transmission.²³ Terminal (liquid) pasteurization at 60°C for 10 hours is the “gold standard” treatment of albumin preparations. The fatty acids, caprylate, and tryptophanate, which protect albumin from heat denaturation, are added

Table 3. Viral Reduction Treatments of Licensed Plasma Products

Treatment	Products	Target viruses in-process treatment	Comments
<i>In-process treatment</i>			
Solvent-detergent	Coagulation factors (eg, FVIII, prothrombin complex, FIX, VWF, fibrinogen) AT IgG Fibrin sealants	E	No, or limited, protein denaturation The SD agents are removed by subsequent protein purification steps
Pasteurization	Coagulation factors (eg, FVIII, fibrinogen) IgG AAT AT	E Most NE	Protein stabilizers may limit viral inactivation B19 is heat resistant 10% to 30% loss of functional activity of coagulation factors
Vapor heat	Coagulation factors (eg, FVIII) C1-inh Fibrin sealants	E Most NE	As pasteurization
Low pH (pH 4) treatment	IgG	E	Most other plasma proteins loose functional activity at low pH
Caprylic acid treatment (<pH 5.5)	IgG and IgM	pH 4 sensitive NE	Most other plasma proteins loose functional activity at low pH
Nanofiltration	Coagulation factors (eg, FIX, FXI, FVIII, VWF) IgG AAT AT Fibrin sealants	E NE	Viral removal by size-exclusion mechanism depends upon virus size and shape, and nanofilter porosity
<i>Terminal treatment</i>			
Pasteurization	Albumin	E NE	Only for a product withstanding liquid heat-treatment in the presence of small amount of stabilizers
Dry heat	Coagulation factors (eg, FVIII, FIX, prothrombin complex, FXI)	Some E Some NE	Inactivation of heat-resistant viruses depends on temperature and duration Hardly inactivates B19 10% to 20% loss of functional activity of coagulation factors

E indicates enveloped; NE, nonenveloped.

at doses compatible with therapeutic use and, therefore, are not removed before product infusion.

Viral removal treatment. Nanofiltration is a specific viral filtration process applied to protein solutions using 15- to 75-nm multi-layers membranes, or equivalent systems, to remove viruses mostly by a sieving mechanism.^{30,31} Introduced in the early to mid 1990s, it has reached wide acceptance as a robust viral removal step for essentially all products, apart from albumin. Nanofiltration is used to complement the core viral inactivation treatment and to provide enhanced safety against nonenveloped viruses or other resistant infectious agents. Virus removal can also

incidentally take place during protein precipitation, chromatography, or filtration steps; these steps contribute to the lowering of virus load from the protein production stream; they are difficult to monitor; and therefore do not guarantee, as stand-alone procedures, sufficient safety margin.

Prion removal methods. Variant Creutzfeldt-Jakob disease (vCJD) can be transmitted by red blood cell concentrates, but to date, transmission has not been identified from plasma or plasma products. Because of its biological nature, the prion agent is thought to be resistant to current viral inactivation procedures used during plasma fractionation. The methods known to inactivate abnormal misfolded

prion proteins associated with transmissible spongiform encephalopathies (PrP^{TSE}) (such as oxidation, treatment with strong base, chaotropic agents, and extreme heat) destroy plasma proteins and, therefore, cannot be used. Still, modern processes of fractionated products would seem to ensure significant removal of PrP^{TSE}, as suggested by experimental spiking studies.³² As scale-down and experimental transmissible spongiform encephalopathy (TSE) spiking models are developed, knowledge on the capacity of manufacturing processes of plasma proteins to remove prions is growing rapidly, although much uncertainty remains because the unknown biological nature of the human plasma associated infectious agent.³³ Several processes for manufacturing FVIII, fibrinogen, von Willebrand factor (VWF), FIX, IgG, and albumin products have been shown to be capable of removing prions in a consistent and reproducible manner.³⁴⁻³⁶ Generally, multistep fractionation processes are thought to be a contributing factor to PrP^{TSE} elimination. Two to more than 5 log removals of spiked prions occur during standard protein purification steps such as precipitation with ethanol or polyethylene glycol (PEG), anion-exchange chromatography, and depth filtration.^{34,35,37} Mechanism of removal that may encompass an adsorption mechanism is still not fully understood and appears to be influenced by pH and the concentration of the precipitating agent. The partitioning process may reflect some prion aggregation because of prion hydrophobicity and insolubility. The removal capacity of nanofiltration membranes with pore sizes less than 75 or 35 nm has been extensively investigated,^{34,38} and prion removal is likely due in part to molecular sieving. Extent of removal is related to the pore size of the filters and, presumably, to the aggregation state of PrP^{TSE}. Removal is superior with membrane of pore sizes of 15 nm, compared to 35 nm. The nature and origin of the spiking agent used for PrP^{TSE} clearance studies is of critical importance because various spikes differ in size and characteristics. Brain homogenate or brain-derived microsomal fractions from infected animals have usually been used as the source of PrP^{TSE} spiking material. The most reliable and quantitative detection method of PrP^{TSE} is based on animal bioassays, which require many animals and a time frame generally longer than 9 months. In vitro immunochemical methods, such as a Western blot assay, are being used, at least as a first marker of the presence of PrP^{TSE} and associated

infectivity, by detecting the protease-resistant fragment.³³ Thus, the risk transmission of vCJD by human plasma products appears remote, but caution should prevail since the biochemical nature of the infectious agent in human blood is not known.

MANUFACTURING PROCESSES OF PLASMA PROTEIN PRODUCTS

Technologies to extract coagulation factors, protease inhibitors, and IgG have evolved considerably in the last 20 years, leading to the development of products with improved safety and purity profiles. A description of major manufacturing techniques is given below.

Coagulation Factors

Factor VIII. Several generations of FVIII preparations have been developed since the mid-1980s, providing (a) safety from HIV and then HCV and HBV, (b) improved purity, and (c) enhanced safety from HAV and B19. Current development efforts focus on establishing prion removal.³⁶ All currently licensed plasma-derived FVIII concentrates are purified from cryoprecipitate. In a typical process, cryoprecipitate is subjected to a combination of aluminium hydroxide adsorption and precipitation, or precipitation only (eg, using glycine), to reduce the level of trace vitamin K coagulation factors (as they may activate FVIII during the downstream purification steps) or load proteins such as fibrinogen. The purified cryoprecipitate extract usually undergoes viral inactivation typically by SD or pasteurization. Many processes include subsequent chromatography by anion exchange, monoclonal antibody affinity (using anti-FVIII or anti-VWF murine antibodies), or immobilized heparin affinity to remove protein contaminants (such as fibrinogen or fibronectin), most or part of the VWF, and the SD agents.³⁹ Immunopurified FVIII eluate is further purified by chromatography to remove murine IgG ligands that may have leached. Prior to formulation and sterile filtration, some FVIII products are nanofiltered using membranes with a pore size of 35, 20, or even 15 nm if a partial dissociation of FVIII and high-molecular-weight VWF multimers is initiated. Alternatively, some freeze-dried preparations are subjected to heat treatment at 80°C or 100°C to inactivate nonenveloped viruses like HAV. Recovery of FVIII, as expressed per liter of plasma, is usually comprised between 100 and 200 IU (1 IU is defined as the physiological activity present in 1

mL of plasma). Factors lowering FVIII yield include cryoprecipitation (ca 30% loss), chromatographic purification (ca 20% to 30 %), and viral heat inactivation (15%-30%). Current FVIII concentrates have a specific activity between 10 and 250 IU/mg. Some products are formulated with human plasma-derived albumin, whereas others contain copurified VWF that helps stabilize FVIII. Purity of FVIII concentrates has not been convincingly demonstrated to enhance immunological safety. Long-term clinical experience indicates that the alleged reduced immunosuppressive effects of immunopurified preparations compared to lower-purity plasma-derived preparations, as claimed in the late 1990s, were probably unfounded. Processes used that, in part, influence residual VWF, may have an impact on the immunogenicity of plasma-derived FVIII products. Retrospective studies of previously untreated patients show that an SD-treated, nano-filtered, ion-exchange purified FVIII product containing VWF appears to be 2 times less likely to induce anti-FVIII inhibitors in hemophilia A patients than 2 full-length recombinant FVIII concentrates.⁴⁰

von Willebrand factor. Because FVIII chromatographic purification removes all, or part, of VWF, FVIII products effective in treating Von Willebrand disease (VWD) are generally low-purity ("intermediate purity") preparations prepared from cryoprecipitate by precipitation steps coextracting VWF and FVIII in a ratio of higher than 1. The low purity and the high protein content of these products technically restrict the choice of viral inactivation treatments to pasteurization or terminal dry heat at 80°C for 72 hours. One highly purified VWF concentrate, largely devoid of FVIII and, therefore, specific for VWD treatment, is prepared from cryoprecipitate by a 3-step chromatographic procedure (integrated with FVIII and fibrinogen purification processes) using 2 anion exchangers and immobilized gelatin polishing (to remove fibrinectin).⁴¹ Viral reduction is by SD, 35-nm nanofiltration, and terminal dry heat at 80°C for 72 hours.⁴²

Fibrinogen. There are 5 registered fibrinogen preparations available for treating a fibrinogenemia or hypofibrinogenemia.⁴³ Traditional preparations are obtained by multiple precipitation steps of plasma or cryoprecipitate using ethanol and glycine, whereas other modern products are purified

by chromatography. Viral reduction is achieved by SD treatment, often complemented by 35-nm nanofiltration or terminal dry heat treatment. Single-step pasteurization at 60°C for 20 hours is used for 1 product.

Fibrin sealants. Fibrin sealants (fibrin glues) comprise fibrinogen-rich and purified thrombin concentrates. When mixing the 2 components, a strong adhesive clot exhibiting hemostatic, sealing, and healing properties is formed almost instantaneously or within a few seconds, offering multiple topical surgical applications. Fibrinogen is prepared by precipitation methods from cryoprecipitate, or from the Cohn fraction I; the fraction may also contain fibronectin, VWF, or factor XIII (FXIII), which may confer other physiological functions.⁴⁴ Fibrinogen fractions are virally inactivated by SD, pasteurization, vapor-heat treatment, and/or nanofiltration. The fibrinogen concentration is typically above 80 g/L and may be formulated in the presence of an antifibrinolytic agent.

Prothrombin complex. Prothrombin complex concentrate (PCC) is a mixture of vitamin K-dependent coagulation factors in which FIX, factor II, and factor X and proteins C and S have a low specific activity between 0.5 and 2 IU/mg.⁴⁵ A few products contain also factor VII (FVII), but usually at levels lower than that of FIX. The manufacture is usually based on a 1960s method that involves diethylaminoethyl (DEAE) Sephadex or DEAE cellulose adsorption of cryo-poor plasma, but downstream ethanol fractions can also be used as starting materials.⁴⁶ Anion exchangers coextract proteins sharing the presence of gamma-carboxyglutamic acid residues, whereas bulk plasma proteins, such as albumin and IgG or AT and AAT remain in the unbound fraction. Precipitation with tricalcium phosphate is used for one product. Viral reduction is most often achieved by SD (TnBP-Tween 80) treatment, complemented by 35- or 15-nm nanofiltration or by terminal dry heat. Pasteurization and vapor heat are applied to 2 products. Viral inactivation treatment by SD requires one subsequent ion-exchange chromatographic step for removal of the virus-inactivating agents. Recovery is in the range of 250 to 380 IU of FIX per liter of plasma.

Single FIX. High-purity FIX products were developed in late 1989,⁴⁷ leading to reduced

risks of thromboembolism, compared to PCC, in hemophilia B patients. FIX is isolated by chromatographic purification of the PCC using anion exchange combined with either immobilized heparin, metal chelate affinity, or monoclonal antibody. These processes yield FIX concentrates with a mean specific activity in the range of 100 to 150 IU/mg and a yield between 200 and 300 IU/L of plasma.

Factor VII. Three specific concentrates rich in FVII and with reduced amount of other vitamin K-dependent clotting factors are currently licensed to control bleeding in deficient patients. The manufacturing process includes ion-exchange chromatography or aluminium hydroxide adsorption, following a downstream procedure similar to that of PCC and FIX. Viral inactivation is achieved by SD treatment, vapor heat, or dry heat.

Factor XI. Two factor XI (FXI) concentrates are currently available for deficient patients. One, of low purity, is purified by chromatography of cryo-poor plasma on DEAE cellulose and immobilized heparin. After freeze-drying, the product is virally inactivated by dry heat. In the other product, FXI is captured by adsorptive filtration then highly purified by cation-exchange chromatography.⁴⁸ This product undergoes dual viral reduction processing by SD and 15-nm nanofiltration.

Factor XIII. Factor XIII is a transglutaminase that catalyzes the final step in the coagulation cascade, cross-linking the loose fibrin polymer into a highly organized structure. The early generation of FXIII concentrates for the treatment of FXIII-deficient patients was extracted from placenta, but 2 plasma-derived products have subsequently been developed. One is purified from a cold-ethanol fraction from cryoprecipitate supernatant, purified by precipitation with sodium citrate and removal of fibrinogen by heating. The product is pasteurized in sorbitol solution, ultrafiltered to remove sorbitol, adsorbed with bentonite, and freeze-dried. The other product is obtained by precipitation steps and is also pasteurized. Factor XIII is also a component of some fibrin sealants. Although still subject to discussion, the presence of FXIII is claimed to contribute to fibrin γ -chain cross-linking and tensile strength, possibly improving the hemostatic effect.⁴⁹

Activated Coagulation Factors. Human thrombin concentrates are available so far only as components of fibrin sealant. Thrombin is prepared by activation of the PCC, usually in the presence of calcium chloride, followed by viral inactivation treatment by SD, purification by cation-exchange chromatography, and viral removal by 15-nm nanofiltration. Final thrombin concentration is usually between 300 and 1000 IU/mL, but preparations with lower potency are available when a slower speed for clot formation of the sealant is preferred for some surgical applications requiring longer time for tissue gluing.

A procedure for large-scale production of a purified plasma-derived FVIIa has been developed in Japan.⁵⁰ FVII is purified by anion exchange and immunoaffinity chromatography and converted to FVIIa by autoactivation on an anion-exchange resin and incubation in the presence of Ca^{2+} for 18 hours at 10°C. This preparation is virally reduced by nanofiltration and dry-heating and is intended for the treatment of hemophiliacs with antibodies against FVIII or FIX.

Anticoagulants and Protease Inhibitors

Antithrombin. Antithrombin concentrates were the first plasma products extracted by affinity chromatography. Production from cryo-poor plasma usually comprises ion exchange chromatography to remove the PCC components, followed by capture of AT on immobilized heparin. Viral inactivation is traditionally achieved by pasteurization in the presence of sodium citrate or a combination of sucrose and glycine, although SD treatment is used as well. Because heat treatment may partially denature AT, a second adsorption step on immobilized heparin can be used to remove altered molecules. Recovery is between 250 and 350 U/L of plasma. Fraction IV-1 is an alternative starting material, but yield is significantly lower.

α 1-Antitrypsin (α 1-protease inhibitor). There are now several licensed AAT concentrates, including 3 in the United States. α 1-Antitrypsin augmentation therapy is indicated for the treatment of patients with lung emphysema secondary to congenital AAT deficiency. Because AAT shares many physicochemical properties, in particular, molecular weight and isoelectric point, with albumin, it has been difficult to design production methods for AAT not affecting the existing

production process for albumin. Most preparations are recovered from fraction IV₁₋₄. Purification from this waste fraction is rather cumbersome and involves PEG precipitation and ion-exchange chromatography, considerably compromising recovery (~0.2 g/L). The first preparations developed in the 1990s were virally inactivated by pasteurization, but dual viral reduction steps using SD and nanofiltration are also now used. Recent isoelectrofocusing studies have provided evidence indicating that new anodal AAT variants are present in at least one of the FDA-licensed product, suggesting alteration of some isoforms during purification. Loss of a C-terminal positive charged lysine, secondary to carboxypeptidase activity, was proposed as an explanation for these isoelectrofocusing migration shifts. Under circumstances when clinical demand for albumin decreases, extracting AAT from upstream fractions, such as the supernatant II+III,⁵¹ appears a logical trend, offering the possibility of a more effective production scheme, characterized by a recovery of 0.6 to 1 g/L.⁵¹

Protein C. There are 2 protein C concentrates manufactured in Europe and 1 in Japan. In one process, the PCC undergoes cascade purification on 3 ion exchangers,⁵² whereas in the other, immunoaffinity and affinity chromatographic processes are combined with ion exchange. Viral reduction is achieved by SD treatment, which can be combined with 15-nm nanofiltration or vapor-heat treatment.⁵³

C1-esterase inhibitor. C1-esterase inhibitor concentrates are used for the treatment of acute phases of angioedema, primarily in the oropharyngeal region and gastrointestinal tract in patients with congenital or acquired C1-inh deficiency. There are 3 products licensed in Europe. Products are generally purified by chromatography from the cryo-poor plasma after extraction of the PCC and, potentially, AT. Viral inactivation is achieved by pasteurization, vapor heat, or SD, possibly combined with nanofiltration.

Albumin

Essentially all therapeutic albumin preparations are prepared by fractionation of cryo-poor (or PCC-poor and/or AT-poor and/or C1-inh-poor) plasma by ethanol fractionation. A critical upstream process step (precipitation II+III) separates the IgG fraction. To optimize recovery, the precipitates generated

during the ethanol fractionation process are separated by depth filtration. Albumin recovery of 75% to 85% (25-28 g/L) and purity of 96% to 98% are typically obtained. Some processes combine ethanol fractionation with a polishing ion exchange chromatography, which generally improves product purity to ca 99%, whereas in one production method, albumin is purified mostly by anion exchange, cation-exchange, and size-exclusion chromatography. The adjustment of the concentration of the purified fraction, typically from 4% to 25%, is achieved by ultrafiltration. The standard viral inactivation method is pasteurization, which, according to most Pharmacopeias, should be performed in the final container rather than on the albumin batch before aseptic filling. Current mean albumin yield is 24 to 26 g/L of plasma.

Immunoglobulin G

Polyvalent IgG preparations, either for intramuscular or intravenous uses, are traditionally prepared from the fraction II that is obtained by stepwise fractionation of cryo-poor plasma using cold ethanol at concentrations up to 25%. Increasingly, IgG products are extracted from up-streamed ethanol precipitated fractions, such as supernatant III or precipitate II+III, to optimize recovery. Intermediate IgG fractions are subjected to ion-exchange chromatography, caprylic acid, or PEG precipitations to remove protein contaminants, proteolytic enzymes, and/or aggregates. Most current viral inactivation procedures are low pH incubation, pasteurization, or SD; the caprylic acid treatment, recently introduced in the manufacture of human IgG products, is also a robust viral inactivation process of IgG. Dedicated viral removal by 15- to 35-nm nanofiltration is commonly used to increase the safety against nonenveloped viruses, especially in a situation when the core viral inactivation treatments target only lipid-enveloped viruses. The IgG recovery has long been in the 2.7- to 3.2-g/L range when combining traditional ethanol fractionation processes and centrifugation. Depth-filtration and/or chromatographic purification from upstream fractions have improved the mean recovery to the 3.5- to 4.5-g/L range, or more. Total chromatographic procedures are increasingly used for the production of hyperimmune IgG products because such processes are amenable to the fractionation of smaller plasma volumes and can optimize

recovery. The manufacturing process includes at least 2 dedicated viral reduction treatments.

Other Potential Plasma Protein Therapies or Indications

Other protein components have been fractionated at pilot-scale and subjected to experimental or human trials. Inter- α -trypsin inhibitor (ITI) is a Kunitz-type serine proteinase inhibitor. Its inhibitory capacity is carried by bikunin, a chondroitin 4-sulfate proteoglycan, which is covalently linked to its heavy chains H1 and H2, but can be released by proteolytic cleavage. An ITI concentrate has been obtained by fractionation of the prothrombin complex on an anion exchanger followed by immobilized heparin and viral inactivation by SD. ITI, as a reservoir of bikunin, may be involved in control of inflammatory processes. In a porcine model of endotoxin shock, ITI improved the hemodynamic, oxygenation, and coagulation parameters.⁵⁴ Administration of ITI very early after the onset of sepsis or repeated injections at later time points (10 and 20 hours) maintains cardiovascular stability and significantly reduces mortality in a rat model.⁵⁵

Transferrin is the major iron binding plasma protein which may prevent cytotoxic effects or predisposition to septic infection due to accumulated free non-transferrin-bound iron when normal iron use is hampered and/or apotransferrin production is decreased. A liquid apotransferrin concentrate has been obtained from Cohn fraction IV by 2 ion exchange chromatographic steps and ultrafiltration. Viral safety was ensured by SD treatment, nanofiltration, and PEG precipitation. The product had intact iron binding capacity, and maintained the bacterial growth inhibitory effect in serum. In hematological stem cell transplant patients, the product prevented the appearance of non-transferrin-bound iron.

Apolipoprotein A-I is the principal protein component of the plasma high-density lipoproteins. It prevents the accumulation of cholesterol-loaded macrophages which deposit on the arterial wall as foam cells. Apolipoprotein A-I inhibits hepatic lipase and lipoprotein lipase in vitro. A concentrate has been isolated by precipitation from Cohn Fraction III.⁵⁶ Intravenous injections in men were well tolerated in an early clinical trial; clinical observations were consistent with combined inhibition of hepatic lipase and lipoprotein lipase

activities. Possible clinical applications include the treatment of hypercholesterolemic patients and atherosclerosis. Recombination with lecithin forms a high-density lipoprotein complex that could help limit inflammation, endotoxin-induced activation of coagulation, and fibrinolysis in septic conditions.

Mannan-binding lectin (MBL) is a component of the innate (aspecific) immune system that can bind repetitive structures of mannan groups, such as those on the surface of micro-organisms, activating the complement system and leading to the destruction of a large variety of micro-organisms. The relatively frequent congenital deficiency of MBL is associated to recurrent infections, especially in infants when the specific immune system has not yet matured. An MBL concentrate has been produced from COHN fraction III.⁵⁷

Plasmin is the major fibrinolytic enzyme in plasma. Encouraging results as a new fibrinolytic agent have been obtained in animal models where plasmin was applied directly to the clot through a catheter to treat peripheral arterial occlusion.

von Willebrand factor cleaving protease (VWF-CP, ADAMTS13) cleaves ultra-large multimers of VWF that enter the blood stream directly after biosynthesis by endothelial cells. If developed, a purified VWF-CP could be useful to treat, in place of plasma, patients with thrombotic thrombocytopenic purpura with congenital or acquired deficiency of VWF-CP.

Activated protein C can be of value for the treatment of sepsis, as demonstrated through the clinical use of a recombinant preparation. There is no therapeutic plasma-derived activated protein C products available yet for therapeutic use, although a process for a highly purified preparation, where cryo-poor plasma is purified by immunoaffinity and anion-exchange chromatographic steps, and SD viral inactivation has been described.⁵⁸

There is also research needed to investigate alternative indications for currently available products. Potential clinical use of C1-inh, aimed at benefiting from its role as inhibitor or attenuator of the activation of complement and contact systems, include septicemia, myocardial infarction, capillary leak syndrome, pancreatitis, and organ transplantation. Intravenous use of a FXIII concentrate was found to increase epidermal growth factor and transforming growth factor- β , suggesting that it may accelerate wound healing of anastomotic leaks and nonhealing fistulas. Factor XIII was also found

to have osteoinductive properties, suggesting use in bone tissue engineering.

REGULATORY ASPECTS

A review on plasma fractionation should also cover the role played by national and international regulatory authorities. Over the last few years, assuring the safety of large-pool plasma products has posed formidable challenges to regulatory authorities and fractionators alike. The complexity of the field, encompassing the diversity in blood and plasma product types and manufacturing processes, made it difficult to enact balanced decisions toward ensuring both product safety and guarantee of supply. Since the 1980s, the agencies regulating the plasma fractionation industry have developed a comprehensive set of measures to ensure the viral safety of plasma products. Multiple layers of regulatory oversight of the plasma industry have been established to ensure overlapping safeguards against the risks of the transmission of blood-borne infectious agents. Several regulations, guidances, position statements have been issued by agencies like the US FDA and the European Medicine Evaluation Agency, which are updated as needed. Those cover important safety aspects required at all stages of the manufacturing chain, from activities at blood establishment preparing plasma for fractionation, extending to the manufacturing and distribution of plasma products. Regulatory oversight includes epidemiologic surveillance of the donor population; donor deferral policies and screening practices; mandatory donation testing; testing of manufacturing plasma pools; validation of viral reduction procedures and other production steps; as well as the assessment of product quality, safety, and efficacy for marketing authorization. Most of relevant information on plasma collection is assembled in Europe in the Plasma Master File, which allows establishing key levels of information regarding the quality and safety of the plasma raw material. Post marketing, reports on adverse reactions associated with plasma products should be transmitted to NRAs and could prompt emergency response procedures and product recalls.¹⁰ Some harmonization of the requirements for manufacture and supply of plasma products in the United States, Europe, and Japan is taking place under the auspices of the International

Conference on Harmonisation, but much work remains to be done. Such measures, nonetheless, provide the framework through which modern plasma products now exhibit a very high level of quality, safety, and efficacy. However, the rigidity of the regulatory system has been an impediment to more significant technological evolution of the plasma fractionation process because process changes are currently associated to major regulatory work.

CONCLUSIONS

The Cohn plasma fractionation method initially designed to obtain albumin has, over the years, developed rather successfully into a well-established industrial procedure isolating a wide range of clinically useful products. Today, more than 20 different protein products, and more if one considers the variety of hyperimmune IgG preparations, can be extracted through large-scale fractionation of human plasma. The soundness and large-scale adaptability of the technology, on the one hand, and the rigidity of the current regulatory framework, on the other, explains why this technology remains the main core method in use at industrial scale, although it implies suboptimal yield for most proteins, apart from albumin. The technology has increased in complexity over the years, with the greatest progresses in purification being, without doubt, associated with the use of chromatographic methods that have made possible the development of new protein therapeutics and impressive improvements in product purity and quality. The fractionation scheme has also changed dramatically through the introduction of in-process viral reduction treatments, which have required the addition of downstream techniques such as chromatography and ultrafiltration.

The change in protein drivers, with the prominent clinical role now played by IgG, and the requirements to increase protein recovery and optimize the fractionation process may crystallize the incentive for most fractionators to abandon relative technical conservatism and introduce significant technological changes in processing technology. The production of IgG from precipitate (I)+ II+III, already implemented by some manufacturers and, possibly, that of AAT from supernatant (I)+ II+III, are signs of a gradual evolution in the direction of total chromatographic processing. With so much gained, over the last few years, in

the understanding of the key parameters building plasma product quality, safety, and efficacy, one can hope that the regulatory paths, in particular, with regard to clinical studies, to license known protein therapeutics prepared by improved, more efficient technology should be simplified. This would, in turn, contribute to an improved supply.

As the plasma fractionation industry has so far been targeting mostly proteins that were obvious candidates for replacement therapy, it is also hoped that it will invest more into developing new products because plasma remains a unique source of potential therapeutic proteins. Proactive research and development work should be encouraged to isolate and evaluate new therapies among the well characterized plasma proteins with still unknown function.

Finally, one should not forget that plasma protein therapies are expensive and largely inac-

cessible to the developing world. The new market drivers in rich countries are likely to diminish economical interest in the manufacture of FVIII that remain a major protein therapy in need in the developing world. The belief that decreased use of plasma-derived FVIII or FIX in developed economies (as hemophiliacs are switching to recombinant therapies) would increase the supply of products to developing countries may be not economically viable. Rather, this switch to recombinant products in rich countries can make poor countries unable to afford the increased cost of products no longer subsidized by the premium price paid in rich countries. It therefore remains important to develop affordable viral inactivation and processing technologies gradually allowing developing countries to make use of local plasma resources in a safe manner.⁵⁹

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REVIEWS

Antibody–Drug Conjugates: Linking Cytotoxic Payloads to Monoclonal Antibodies

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Antibody–drug conjugates (ADCs) combine the specificity of monoclonal antibodies (mAbs) with the potency of cytotoxic molecules, thereby taking advantage of the best characteristics of both components. Along with the development of the mAbs and cytotoxins, the design of chemical linkers to covalently bind these building blocks is making rapid progress but remains challenging. Recent advances have resulted in linkers having increased stability in the bloodstream while allowing efficient payload release within the tumor cell.

INTRODUCTION

After cardiovascular diseases, cancer represents the second most common cause of death in the Western world and is a major area of focus for the pharmaceutical industry. Surgery and radiotherapy are generally used when a tumor is localized to a certain tissue, but chemotherapy is needed when metastasis has occurred. Despite extensive research, most anticancer drugs have nonspecific toxicity. By targeting the cell cycle and thereby killing rapidly proliferating cells, they do not explicitly discriminate between healthy and tumor tissues and only gain a limited selectivity for malignant cells. Such cytotoxic drugs have a narrow therapeutic window, which limits their efficacy and results in severe side effects. Due to a lack of selectivity, drug concentrations that would eradicate the tumor can often not be used. In addition, tumors can develop resistance against anticancer drugs after prolonged treatment. Therefore, achieving improved tumor selectivity through targeting of cytotoxic drugs to the cancer cells is needed.

A promising approach to achieving a more selective treatment is targeted prodrug therapy (1). Antibody–drug conjugates (ADCs) are ideal candidates for such prodrugs. ADCs are monoclonal antibodies (mAbs) linked to cell-killing drugs. Thanks to their high binding specificity for tumor-specific antigens, mAbs can be used as vehicles to target cell-killing payloads to tumor cells. Unique or overexpressed, tumor-specific antigens can be found in a wide range of human tumor cells (2). Some mAbs have the ability to recognize and specifically bind to these tumor-associated antigens. They can be used as single agents for the treatment of cancer through binding to cancer-cell-specific antigens and induction of an immunological response against the target cancer cell (3). However, therapeutic efficacy is often limited by the extent to which the antibody leads to cell death. Monoclonal antibodies are extremely discriminating for their targets but sometimes therapeutically ineffective on their own. The insufficient efficiency of most naked mAbs in cancer therapy has been circumvented by arming the immunoglobulin with radioactive isotopes (4) or cytotoxic drugs (5–7) (Figure 1), yielding highly specific ADCs.

ADCs can be viewed as sophisticated delivery systems for antitumor cytotoxic drugs. The mAb guides the toxin precursor

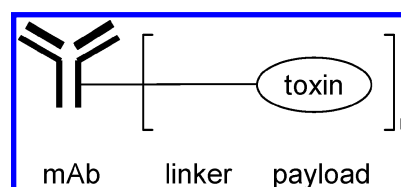


Figure 1. Schematic representation of an antibody–drug conjugate.

to the target cancer cell, where the prodrug can be converted chemically and/or enzymatically to the parent drug and unfold its cytotoxic activity. The ADC is exposed to different conditions on its journey from the blood vessels to the molecular target in the tumor tissue. The mode of action at cellular or molecular level is complex, with each step bringing its own challenges:

a. Circulation. The ADC must behave like a naked antibody when circulating in the plasma. In particular, the linker must be stable in the bloodstream to limit the damage to healthy tissue. Decomposition or decay would release the cytotoxin before being delivered to the target site.

b. Antigen Binding. It is necessary that the conjugated mAb retains high immunoaffinity. Attaching the cytotoxic compound to the mAb must thus not disturb its binding specificity.

c. Internalization. A sufficient intracellular concentration of the drug must be achieved. This is challenging because antigen targets on cell surfaces are often present in limited numbers and the internalization process for antigen–antibody complexes is frequently inefficient.

d. Drug Release. Once internalized, the ADC has to efficiently release the original cytotoxic drug in its active form inside the tumor cell.

e. Drug Action. The inherent potency of the released drug must be sufficient to kill the tumor cell, even at low concentration. To achieve significant cytotoxicity, use of very potent drugs with subnanomolar IC_{50} (as free drug) becomes necessary. It has been shown that compounds found to be too toxic when tested as a stand-alone chemotherapy agent are suitable candidates for ADC payloads. These toxins can be 100 to 1000 times more cytotoxic than traditional anticancer agents.

The molar loading of the drug on the mAb can be tuned to achieve the desired potency. Because of points (c) and (d), linkage of several drugs is generally necessary to achieve significant cytotoxicity. However, if too many cytotoxic molecules are attached

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Chart 1

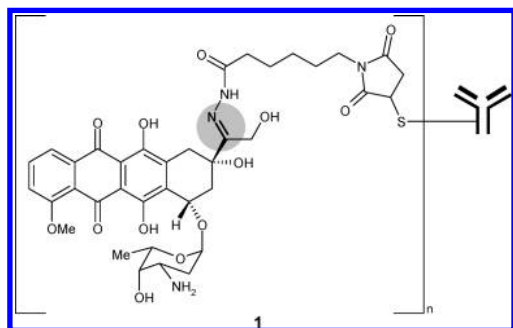
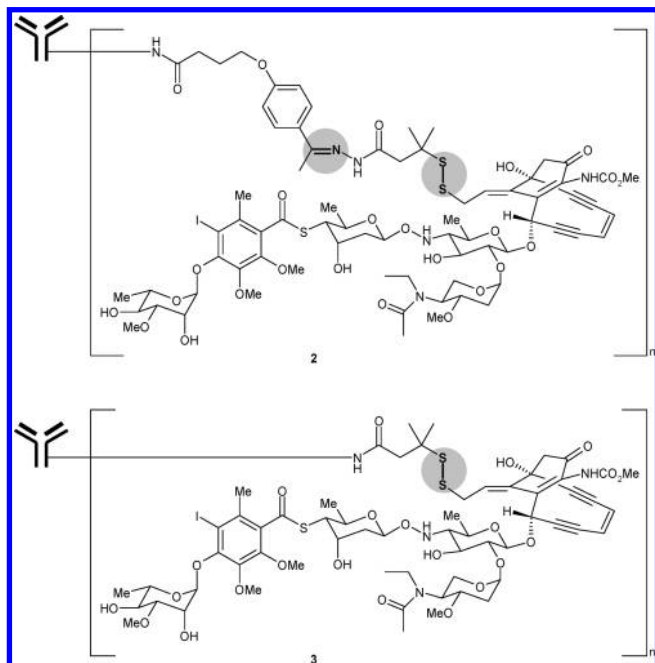


Chart 2



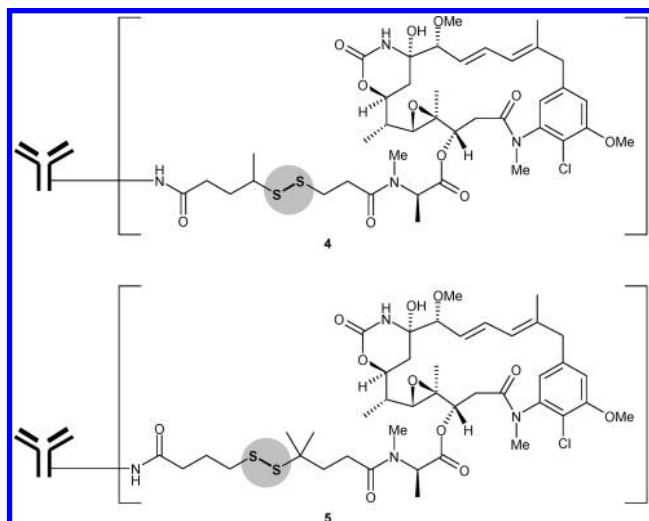
to the antibody, the body may recognize the conjugate as a damaged form of the protein and quickly clears it from the system, proving points (a) and/or (b) to be problematic. The extent of drug substitution may also affect pharmacokinetics, so that most of the time 2–4 drugs per antibody give the best therapeutic window (8, 9). To avoid the interference with the antigen recognition, the drugs are ideally linked at the Fc or constant region of the mAb that does not participate in binding to the antigen (point (b)). To regain the full cytotoxic potential of the drugs, cleavable linkers releasing the drug molecule at the target site in unmodified form were developed to address point (d), although these also affect the stability of the construct. Therefore, the right balance between plasma stability and efficient drug release at the target cell has to be found.

The insights gained during ADC development within the past decade have resulted in a number of new strategies and drugs (4–7). This review focuses on linker technologies used to attach low molecular weight cytotoxic drugs to mAbs. The linker is a small but central part of ADCs and as such accounts for stability in circulation, favorable pharmacokinetic properties, and efficient release of toxic agent at the tumor site.

CHEMICALLY LABILE LINKERS

Selective linker cleavage and payload release is based upon the differential properties between the plasma and some cytoplasmic compartments. Linkers were chosen that were stable in the blood's neutral pH environment but were getting cleaved

Chart 3



once the ADC entered the lower pH environment found inside a cell. The focus in the early days of ADC development was on acid-cleavable hydrazone linkers that were relatively stable at neutral pH (bloodstream pH 7.3–7.5), but were undergoing hydrolysis once internalized into the mildly acidic endosomes (pH 5.0–6.5) and lysosomes (pH 4.5–5.0).

In the 1990s, Bristol-Myers Squibb scientists covalently linked doxorubicin, an intercalating agent that blocks DNA replication and is used as chemotherapeutic agent, to the humanized mAb BR96 (10). Doxorubicin was connected over a (6-maleimidocaproyl)hydrazone linker to cysteine residues of the Lewis-Y specific mAb. The preclinical activities of BR96-doxorubicin (**1**, Chart 1) were remarkable, but despite the use of eight drugs per mAb molecule, the amount of conjugate needed to achieve these effects *in vivo* were high (>100 mg/kg). This was presumably due to the relatively low potency of doxorubicin (IC_{50} of 0.1–0.2 μ M for human carcinoma lines (11), whereas subnanomolar activities are now typically seen for ADC payloads). Moreover, the half-life of the drug in the blood was approximately 43 h, which is short compared to the half-lives of several days to weeks of the naked BR96 mAb in humans. The compound later failed to show sufficient clinical efficacy.

The first and so far sole mAb linked to a cytotoxic payload that has been given regulatory approval is Wyeth's gemtuzumab ozogamicin (**2**, Mylotarg; Chart 2) (12–14). The drug was approved by the US Food and Drug Administration (FDA) in 2000 for use in patients over 60 suffering from relapsed acute myelocytic leukemia (AML), the most common form of leukemia in adults. Gemtuzumab ozogamicin (**2**) consists of *N*-acetyl- γ -calicheamicin (an enediyne antibiotic that binds to the minor groove of DNA and causes double-strand breaks leading to apoptosis) covalently attached to humanized anti-CD33 IgG4 κ antibody (hP67.6) via a bifunctional linker. The 4-(4-acetylphenoxy)butanoic acid moiety allows for attachment to surface-exposed lysines of the antibody over an amide bond and forms an acyl hydrazone linkage with *N*-acetyl- γ -calicheamicin dimethyl hydrazide. Upon internalization of the ADC, the calicheamicin prodrug is released by hydrolysis of the hydrazone in lysosomes of the CD33-positive target cells. The hydrolyzability of an unconjugated intermediate at 37 °C over 24 h increased from 6% at pH 7.4 to 97% at pH 4.5 (15). The enediyne drug then gets activated by reductive cleavage of the disulfide bond. This disulfide linkage has been stabilized by two methyl groups to prevent premature release of calicheamicin by circulating reduced thiols, such as glutathione. In most preclinical models, the hydrazone linkage produced ADCs with higher potency than the corresponding amide-bearing conjugate **3**, providing evidence that with this mAb the disulfide alone is insufficient for

Scheme 1. Presumed Release Mechanism of MMAE (7) from ADC 6 Incorporating a Valine-Citrulline Peptidic Linker

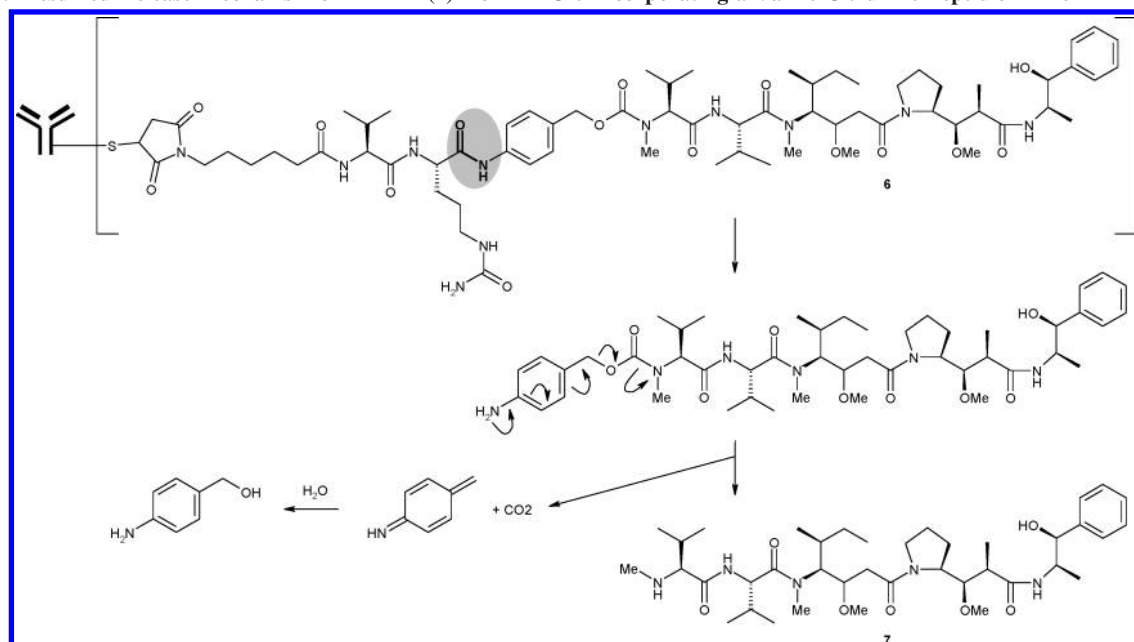


Table 1. Compared Stabilities of Enzyme-Labile and Chemically Labile Linkers in ADCs with MMAE Conjugated to cBR96 mAb

ADC	Linker	Half-live mouse plasma	Half-live human plasma	Maximum tolerated dose
	Valine-citrulline	30 days*	230 days*	30 mg/kg
	Phenylalanine-lysine	12.5 days*	80 days*	n/a
	Hydrazone	2.1 days	2.6 days	15 mg/kg
* projected half-life				

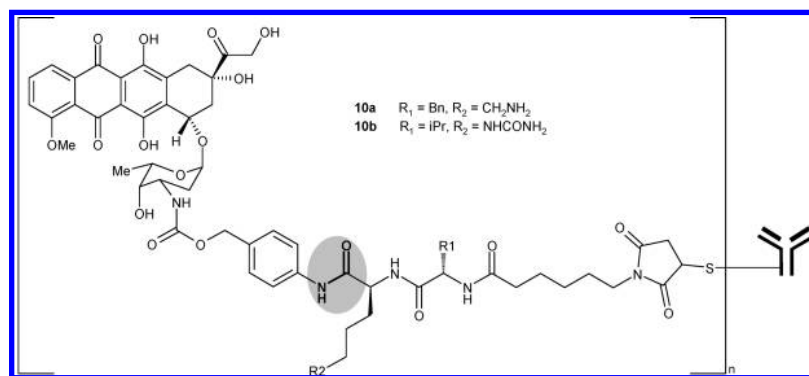
efficient release of the drug in the target cell. Interestingly, with the murine mAb CTM01, ADC **3** showed activities equal to or even greater than that of the corresponding hydrazone conjugate **2** in several *in vitro* and *in vivo* tumor models, revealing that this conclusion is not general (16). In clinical trials, however, compound **3** had only limited evidence of activity.

Mylotarg has met limited successes due to a narrow therapeutic window. The linker technology based on pH-dependent release mechanism is possibly not sufficiently stable and too much of the drug is being released in the bloodstream, as pharmacokinetic data have shown that the mean half-life of Mylotarg is 72 h (15). Nonetheless, the development of CMC-544 (inotuzumab ozogamicin), which is a humanized anti-CD22 mAb identically attached to *N*-acetyl- γ -calicheamicin dimethyl

hydrazide via the acid-labile 4-(4'-acetylphenoxy)butanoic acid linker is ongoing at Wyeth. Although this ADC is closely related to Mylotarg, good stability was shown in both human plasma and serum (rate of hydrolysis of 1.5–2%/day over 4 days) (17, 18).

Disulfide bonds can be an alternative to acid-labile hydrazone linkers. In this case, the release is attributed to the high intracellular concentration of glutathione. This thiol-containing tripeptide is present in micromolar concentrations in the blood, whereas its concentration in the cytoplasm is in the millimolar range (up to 1000-fold higher). This is especially true for tumor cells, where irregular blood flow leads to hypoxic state (decreased oxygen level), resulting in enhanced activity of reductive enzymes and therefore even higher glutathione concentrations. In the absence of sulfhydryl groups, disulfide

Chart 4



bonds are thermodynamically stable and provide reasonably good stability in the bloodstream.

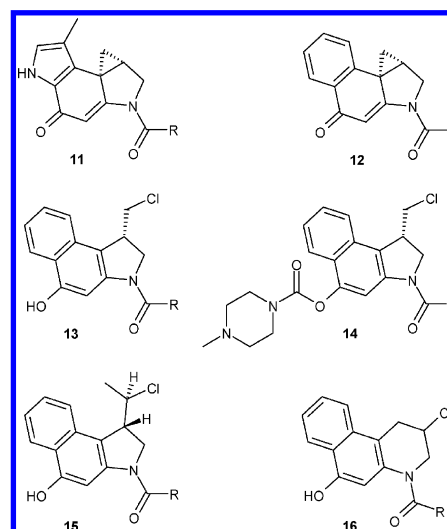
An example of an intracellularly cleavable disulfide-based linker was already discussed above (see “amide” conjugate **3**). Other examples are taxoid ADC payloads, conjugated via a disulfide-bearing 4-mercaptopentanoate linker to mAbs directed against the epidermal growth factor receptor (19). However, the most significant examples are ADCs where the immunoglobulin is connected over the same type of linker to ImmunoGen’s drugs DM1 or DM4 (20–22). These potent thiol-containing maytansine analogues are antimitotic agents that bind to tubulin and inhibit microtubule assembly. After internalization of the ADC via antigen-mediated endocytosis and delivery to lysosomes by vesicular trafficking, the mAb is believed to be degraded, affording lysine derivatives linked to the toxin (22). Further intracellular modifications like cleavage of the disulfide linker through a disulfide exchange, and finally thiol methylation presumably catalyzed by intracellular methyltransferases, generate potent derivatives of DM1 or DM4 (22). These methylated drugs are uncharged, enabling them to pass back out through cell membranes to broaden their attack; the drug not only kills from inside the cell, but also knocks out adjacent cells within the tumor that are not targeted by the antibody (23). The linker of compound **4** (Chart 3) nevertheless proved to be relatively unstable *in vivo* (half-life of 47 h in mice) (24). Replacement of DM1 by DM4 structurally leads to the introduction of two methyl substituents on the carbon atom geminal to the disulfide bond, thereby limiting the accessibility for reducing agents. Consistent with these considerations, ADC **5** exhibits an increased half-life of 102 h in mice.

ENZYME-LABILE LINKERS

Chemically labile linkers often suffer from limited plasma stability. An alternative approach to achieve better control of the drug release involves linking a drug to an antibody via a peptide linkage. The free drug can be specifically cleaved from the carrier by the action of lysosomal proteases (e.g., cathepsin or plasmin) present at elevated levels in certain tumor tissues (25). Peptidic bonds are expected to have good serum stability, as proteases are normally not active outside cells because of unfavorable pH conditions and inhibition by serum protease inhibitors.

Seattle Genetics is using a maleimido-containing dipeptide linker to conjugate cysteine residues of mAbs with monomethyl auristatin E (MMAE, **7**) and F (MMAF), two auristatin derivatives that bind to tubulin and inhibit microtubule assembly. A valine-citrulline linker was designed to provide high plasma stability (half-lives in mice and monkey of 6.0 and 9.6 days, respectively) as well as a cleavage site for proteases (26). Efficient hydrolysis of the dipeptide linker by cathepsin B has been established *in vitro*. After enzymatic

Chart 5



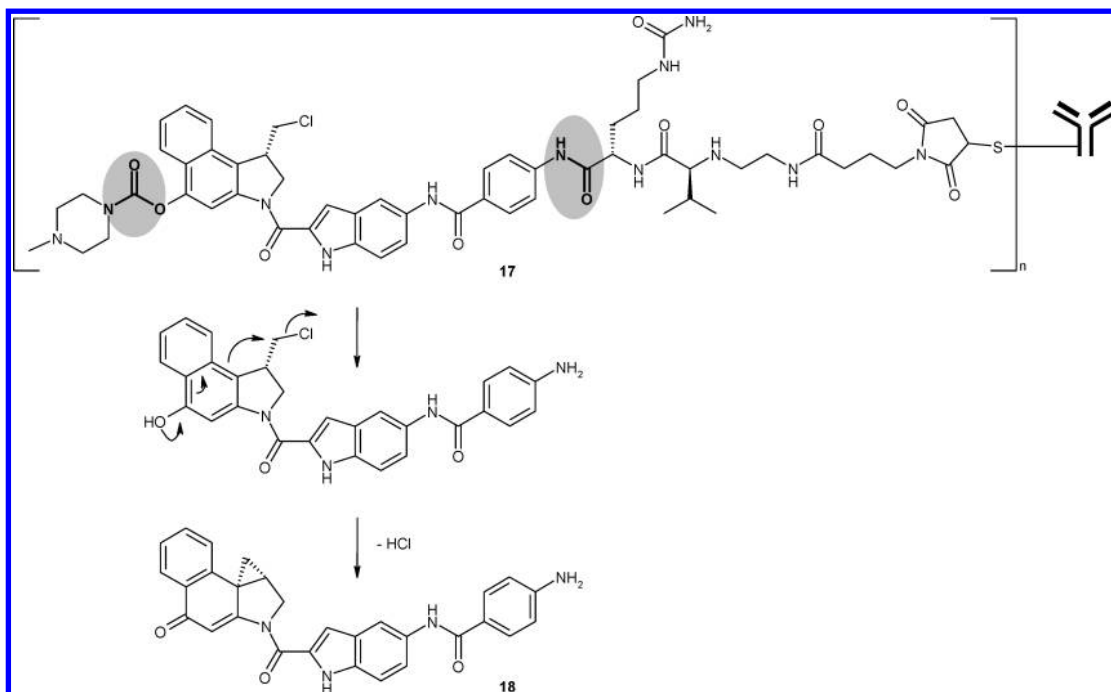
cleavage, 1,6-elimination of the strongly electron-donating 4-aminobenzyl group will occur, releasing MMAE in its active form (Scheme 1). This self-eliminating spacer is needed to spatially separate the drug from the site of enzymatic cleavage (27). Otherwise, the bulky payload has a negative influence on the kinetics of the peptide hydrolysis, as the access to the enzyme’s active site can be affected.

Comparison of plasma stabilities of ADCs bearing enzyme-labile valine-citrulline or phenylalanine-lysine linkers (**6** and **8**, respectively) with ADC **9** equipped with a chemically labile hydrazone linker connecting MMAE with the cBR96 mAb showed impressively the advantage of the peptide linkage (Table 1): in human plasma, the ADC **6**, featuring a peptidic valine-citrulline-derived linker, exhibits an almost 100-fold increased stability compared to its hydrazone relative **9** and the *in vitro* specificity was increased (26). The peptidic linker has a half-life of 6 days in mice compared to 2 days for the hydrazone linker (3-fold improvement), resulting in a lower *in vivo* toxicity (28). Several ADCs incorporating a peptidic linker are in clinical development, with the most advanced being cAC10-vcMMAE (SGN-35) for CD30-positive hematologic malignancies (26, 29, 30).

In addition to *N*-terminus linked MMAE and MMAF, attachment of dipeptide linkers at the *C*-terminus was studied as a way to vary the rate of drug release and thus ADC potency and tolerability (26, 31). For MMAF, it could be shown that linking the mAb over a dipeptide linker to the *C*-terminus of the drug can lead to further improvement of the therapeutic window.

The superiority of enzyme-labile linkers was also shown for doxorubicin derivatives conjugated to mAbs (11, 32). With this

Scheme 2. Presumed Release Mechanism of CBI-Containing Drug 18 from ADC 17



strategy, the immunological specificity of BR96 mAb–peptide–doxorubicin conjugates **10a** and **10b** (Chart 4) was significantly improved compared to that of the corresponding hydrazone-based conjugate **1** (11), demonstrating again the importance of the linker structure for the ADC properties.

Peptide-containing linkers have been successfully used with CC-1065-derived toxins. CC-1065, duocarmycins, and other cyclopropapyrrolind-4-one (CPI, **11**; Chart 5) derivatives are potent minor-groove binding DNA alkylating agents. According to Boger et al., cyclopropabenzindol-4-one analogues (CBI, **12**) are chemically more stable, biologically more potent, and synthetically more accessible than their parent compounds incorporating the natural CPI alkylation subunit (33). In analogy to a duocarmycin prodrug (34), the seco-CBI precursor **13** was developed. This compound, which was postulated to show improved pharmacokinetics, will provide the CBI analogue **12** via Winstein cyclization (35). A further improvement was achieved by protecting the phenolic hydroxyl group with an enzyme-labile carbamate, as in derivative **14**, decreasing the toxicity of the prodrug while increasing its water solubility by introduction of an additional basic nitrogen (36).

Using their UltiMAB technology, Medarex conjugated compound **14** to a mAb via a peptidic linker (37). Conceptually, this approach is a double prodrug strategy. It does not only improve the drug's therapeutic efficacy by conjugating it to a tumor-specific mAb, but additionally employs a prodrug of the cytotoxic agent that has reduced potency and thus minimizes side effects before being released intracellularly by carboxyesterases. The two activating steps needed to trigger the release of the active drug (Scheme 2) may lead to an enlarged therapeutic window.

Tietze et al. have reported that the CBI prodrugs **15** and **16** exhibit lower toxicities due to the reduced reactivity of the secondary chloride compared to the primary chloride present in **13** and **14** (39–41). Such derivatives with secondary chlorides (see, for example, ADC **19**; Chart 6) are used in Syntarga's SpaceLink conjugation technology (42). To our knowledge, no data are yet published on the linker stability.

To extend its ADC technology to drugs with a complementary mode of action, Seattle Genetics has developed

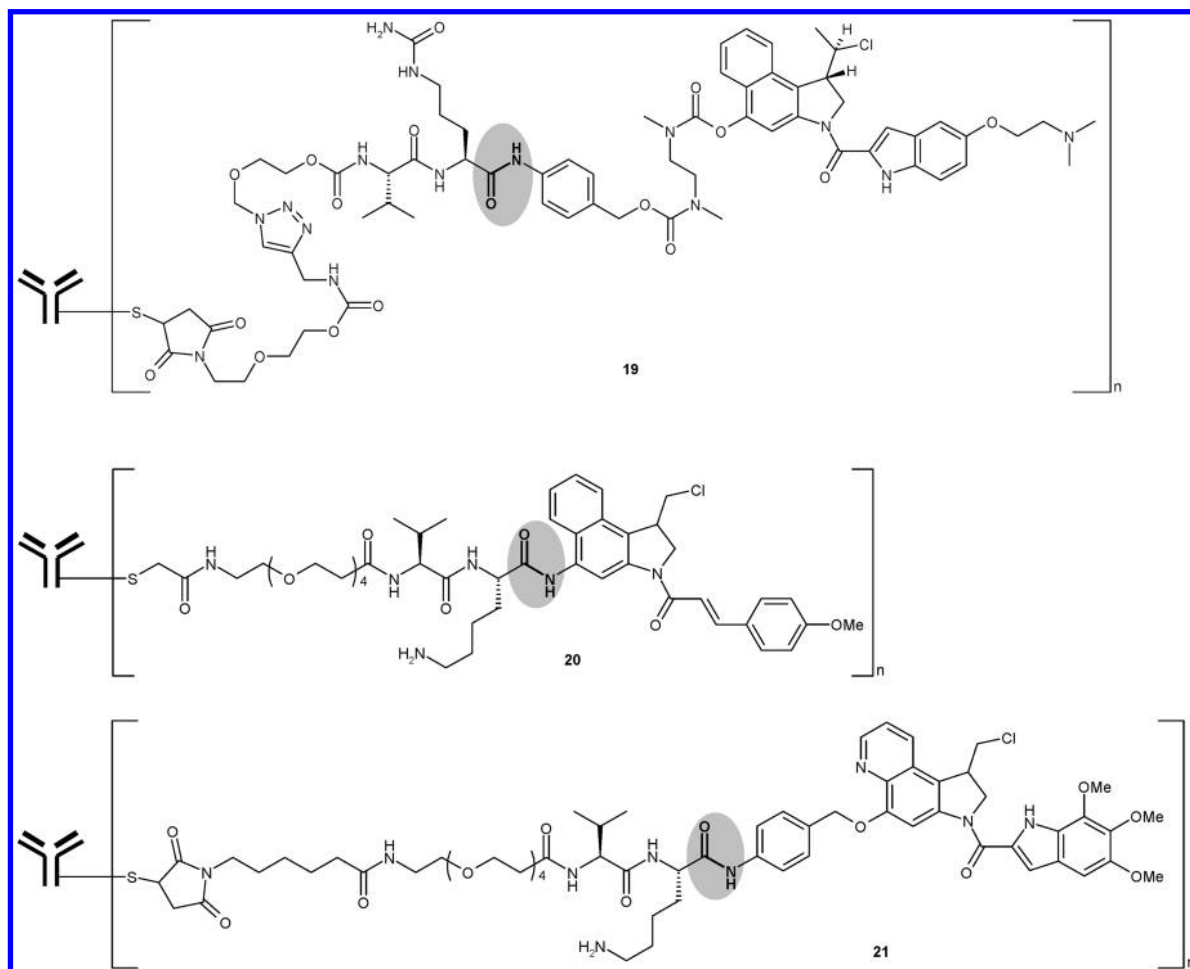
ADCs **20** and **21** (Chart 6) containing an amino-CBI and a hydroxy aza-CBI payload, respectively (43). Because of the hydrophobicity of this class of molecules, attention was focused on developing hydrophilic peptide-linker derivatives that prevented aggregation. This was achieved by use of a more hydrophilic valine-lysine sequence (compared to valine-citrulline as in ADC **6**) and incorporating a tetra(ethylene glycol) unit between the mAb and the peptide linker. The direct attachment of the linker to the amine of the CBI building block (as in **20**) or over a self-immolative *p*-aminobenzyl spacer to the corresponding hydroxyl group of the aza-CBI toxin (as in **21**) prohibits the spontaneous formation of the active toxin in the plasma. Only the enzymatic cleavage of the linker after internalization of the ADC into the cancer cell triggers the release of the prodrug that can now be transformed into the DNA-alkylating cyclopropyl derivative via a Winstein cyclization.

Another type of enzyme-labile linker was recently reported by Seattle Genetics (44). In this case, a carbohydrate moiety serves as the cleavage site. Its hydrophilic nature should limit aggregation. Interestingly, the β -glucuronide moiety in ADC **22** is not directly linking the ADC with the payload. Nevertheless, cleavage by β -glucuronidase triggers the 1,6-elimination of the spacer liberating the free drug **23** (Scheme 3). This carbohydrate motif was also used in ADCs bearing Camptothecin analogues (45).

NONCLEAVABLE LINKERS

ImmunoGen has reported the fortuitous discovery of a noncleavable thioether bond to link DM1 to mAbs (22, 46). Derivative **24** was actually prepared as a control experiment, but surprisingly, this noncleavable thioether linked ADC also proved very potent. The release mechanism is believed to occur via internalization of the ADC followed by degradation of the mAb component in the lysosome, resulting in the release of the maytansinoid drug **25** still attached via the linker to a lysine residue. This chemical modification of the drug did not diminish its cytotoxic potential. This form of the drug is, however, charged and presumably not able to diffuse into neighboring cells. Hence, it cannot kill adjacent tumor cells

Chart 6



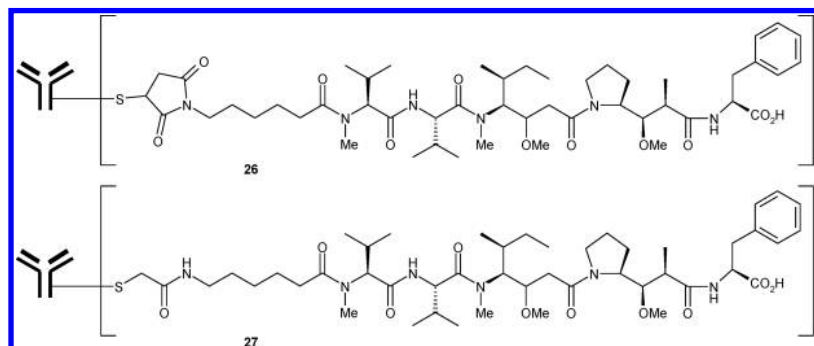
that do not express the target antigen (antigen-negative cells). Besides this by-stander effect (23), there is an additional reason why cleavable linkers may have a broader efficacy. Although internalization is often the common initial activation process for both cleavable and noncleavable linkers (22), ADCs with cleavable linkers may also be active even when they are poorly internalized (47). One possible explanation is that, when the linker is cleaved in extracellular space, the free drug subsequently permeates the cell to reach its target. This mechanism is not operating for ADCs incorporating noncleavable linkers, which must be internalized and degraded within the cell to become active. They are thus dependent on the biology of the target cell.

The *in vivo* stability of the thioether-linked ADC **24** proved, with a half-life of 134 h in mice, superior to that of compounds **4** and **5** (47 and 102 h, respectively). ADCs with

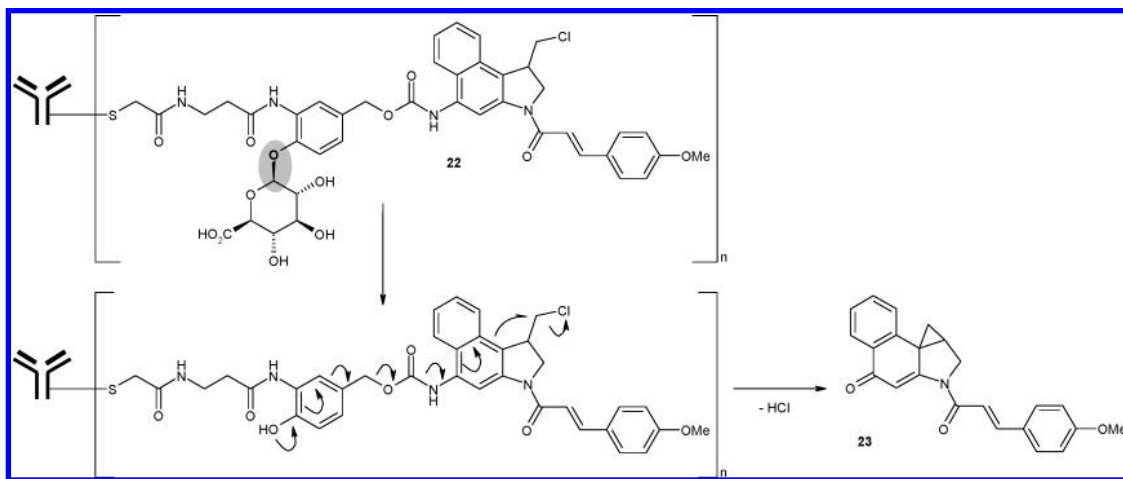
noncleavable linkers were found to be better tolerated (47), presumably resulting in an improved therapeutic index. The use of noncleavable linkers has thus become an important feature of ImmunoGen TAP (tumor-activated prodrug) conjugation technology. This is best exemplified by the promising clinical results obtained by Genentech's trastuzumab-DM1 (T-DM1) for HER2-positive metastatic breast cancer (48).

Seattle Genetics made the same finding with MMAF, namely, that the cleavable dipeptide linker (as in **6**) could be replaced by a noncleavable thioether bond (as in ADC **26**; Chart 7) while retaining the *in vivo* activity (49). Mass spectrometry showed that the released drug was a cysteine-adduct of MMAF, presumably resulting from mAb degradation within lysosomes as proposed for maytansinoid conjugate **24**. MMAE, although closely related structurally, was not

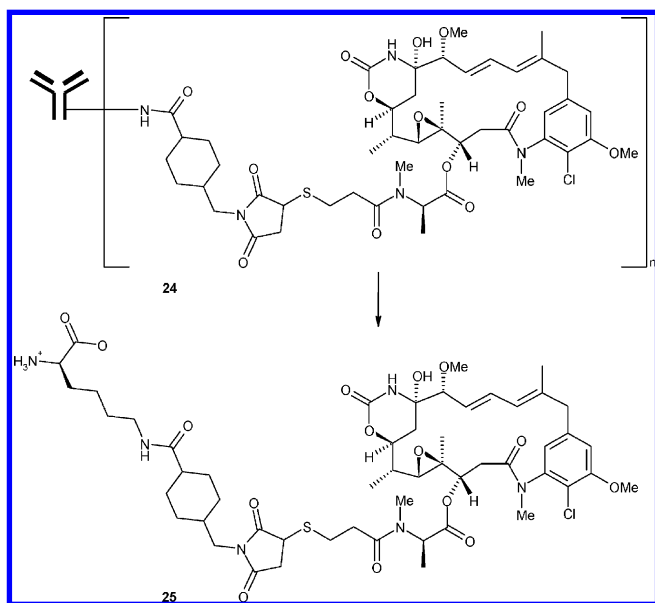
Chart 7



Scheme 3. Postulated Release Mechanism of CBI-Containing Drug 23 from ADC 22



Scheme 4. Release of Maytansine Drug-Derivative 25 through Lysosomal Degradation of ADC 24



active when attached in this manner. Only MMAF can sustain significant modification to the *N*-terminal position and still retain its potency. Recently, it was shown that a slow transfer of the drug from the ADC to albumin cysteines in the plasma occurs with alkyl-maleimide ADCs (50). To counteract this effect, the linker stability was enhanced by replacing the maleimide (as in ADC 26) with an acetamide functionality (as in ADC 27). This type of ADC led to no apparent degradation in the bloodstream for up to 14 days.

CONCLUSION

A promising approach to improve the potency of antibody-based cancer therapies is to conjugate the mAb with cytotoxic chemotherapeutic drugs. In the foreseeable future, ADCs will eventually fulfill the promise of specific delivery of cytotoxic drugs to tumor cells (targeted therapy), thus avoiding the dose-limiting toxicity of chemotherapy that occurs as a result of its effects on normal cells. The linker technology profoundly impacts ADC potency, specificity, and safety. Early ADC linkers were commonly derived from acid-labile hydrazones, which were designed to be cleaved inside target cancer cells, but inevitably underwent cleavage at nontarget sites. Newer linkers feature hindered disulfides and peptidic

moieties that have achieved greater *in vivo* stability. To spare nontarget tissues from chemotherapeutic damage, noncleavable linkers were recently developed. They are thus far the most stable linkers used in ADCs. This recent development may shift some of the future challenge in ADC development from the linker technology toward finding cytotoxic drugs that retain their potency when bearing a covalently attached linker. The improved linker stability and reduced toxicity, however, come together with greater target dependence than in the case of cleavable linkers. ADCs with noncleavable linkers must be internalized and degraded within the cell, whereas compounds with cleavable linkers may be active against targets that are poorly internalized through extracellular drug release and drug entry into tumor cells. Similarly, killing of bystander antigen-negative cells through targeting of antigen-positive cells (collateral toxicity) is presumably only possible with cleavable linkers. The Lonza ADC-team thus believes that no general linker design exists for all ADCs or cancer types. Despite significant progress, each mAb must be examined separately in order to optimize a specific delivery system.

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