The Pharmaceutical and Chemical Journal, **2018, 5(4):10-17**

Available online www.tpcj.org

Review Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

The Fundamental Role of Chromatography in the Large Scale Manufacture of Plasma-Derived Medicinal Products

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Abstract Plasma-derived medicinal products (PDMPs) are valuable group of medications which are used for prevention, control and treatment of several life-threatening diseases. Today, due to the increasing demand for PDMPs, the industrial scale production of these medicinal products has modified from those mainly based on cold ethanol fractionation, to combined consecutive processes including cryoprecipitation, ethanol fractionation, chromatographic procedures and pathogen reduction methods. However it has been proved that the implementation and combination of selective and specific chromatographic procedures in the plasma fractionation industry will be accompanied by main advantages such as improvement of product purity, safety, and quality, optimization of protein recovery, increasing the diversity of plasma products, removal of unwanted compounds and improvement of cost-effectiveness of plasma processing. Thus in this review some of the upstream or downstream applications of chromatographic procedures in extraction, isolation, purification, manufacture and analysis of Plasma-derived medicinal products will be briefly discussed**.**

Key words: Chromatography, Fractionation, Manufacture, Plasma-derived medicinal products

Introduction

Plasma-derived medicinal products

Plasma-derived medicinal products (PDMPs) are valuable class of therapeutics, used for prevention, management and treatment of coagulation factor deficiencies, metabolic and thrombotic disorders, immunological diseases, infections and several other life-threatening conditions [1]. Moreover, these products are widely used in the manufacture of other medicinal products and in coping with bioterrorism [2].

Due to the increasing current needs for PDMPs like Albumin, Coagulation Factors, Immune Globulins, Anticoagulants, protease inhibitors and growth factors [3]. Utilization of optimal, efficient and economical production methods is very important to improve the quality, purity, safety, yields and diversity of these medicinal products.

Ethanol fractionation, the oldest process for large scale protein purification, developed about 70 years ago by Cohn and Co-workers [4,5]. This method, based on the manipulation of PH, ionic strength, ethanol concentration and temperature, has retained as the backbone of industrial plasma processing for the extraction of therapeutic plasma products. Simplicity, High volume of plasma fractionation capacity, safe history of method and its products, and relatively low cost are some of advantages of Cohn fractionation process.

However, today the industrial scale manufacturing of PDMPs has evolved from those primarily based on cold ethanol fractionation to integrated hybrid processes including cryoprecipitation, ethanol fractionation, chromatographic procedures and pathogen reduction technologies, which complement each other and increasethe purity, efficiency, safety and yields of products [6].

Chromatographic methods in plasma fractionation industry

Both preparative and analytical chromatographic procedures have played a crucial role in the large scale production of pharmaceutical and biological products [7,8].Chromatography has been accepted for the extraction of plasma derivatives since the early 80s and today, it is used in the industrial manufacture of PDMPs, as an extension to existing cold ethanol fractionation. Improvement of product purity, safety, and quality, optimization of protein recovery, increasing the diversity of plasma products, removal of unwanted additives and improvement of cost-effectiveness of plasma fractionation, are some of main advantages of using chromatography in plasma fractionation industry [1,9]. In addition chromatographic techniques have been extensively used for the analysis of fractionated plasma products [8]. Moreover, the important role of chromatography should not be forgotten in the removal of plasma-borne viruses, unwanted chemicals and protein contaminants. Therefore implementation of selective and specific chromatographic procedures is of particular value in extraction, isolation, purification, manufacture and analysis of PDMPs.

Thus, considering the growing demand for PDMPs, it can be predicted that the use of different chromatographic methods such as Ion Exchange Chromatography, Affinity Chromatography and Size Exclusion Chromatography, alone or in combination, become more significant in the plasma fractionation industry in the future [2,9,10].

Ion Exchange Chromatography *(IEX)*, which utilizes ionic and surface charge differences in proteins, is probably the most widely used chromatographic method for the separation and purification of plasma proteins[1,7,10,11]. This method may be used either upstream for extracting the desired proteins, or downstream as a polishing step for eliminating unwanted compounds [9].

Affinity Chromatography, which uses a unique specificity between a ligand and the protein of interest, is a highly selective and powerful technique for production and purification of PDMPs too, particularly those present in trace amounts. This method and its sub-categories may also be used either upstream to capture the desired proteins, or downstream as a polishing step for removing trace contaminants [1,10,12].

Size Exclusion Chromatography (SEC) separates proteins on the basis of differences in molecular size and architecture and can be useful in plasma fractionation as a terminal polishing step to eliminate protein contaminants, to remove salts from proteins and to separate aggregates from monomers and dimmers [1,7,9-11,13].

The following examples, point out to some of the above-mentioned chromatography methods, used in the plasma fractionation industry with specific objectives.

Product manufacturing and purification

Today, there is a growing usage of different chromatographic methods in the large scale production and further purification of PDMPs. One of these products on the World Health Organization's List of Medicines, is Factor VIII concentrate, also known as anti-hemophilic factor. The initial step for manufacturing this product is Cryoprecipitation [1]. However, anion exchange chromatography [1,14,15], affinity chromatography [16-18], and to a lesser extent size exclusion chromatography [15], and immobilized heparin affinity chromatography [19,20],a re used, alone or in combination, for product purification [1,2,10,12].

Another plasma derived medicinal product, obtained by chromatographic purification of cryo-poor plasma, is F IX Concentrate [12]. Typical chromatographic methods in the manufacture of F IX concentrates are Ion-exchange chromatography[21,22], affinity chromatography and immunoaffinity chromatography [1,21-25]. However, high purity F IX products can be extracted from cryo-poor plasma by combining IEX chromatography with immunoaffinity chromatography, immobilized heparin affinity chromatography, immobilized metal chelate affinity chromatography (IMAC), or monoclonal antibody chromatography [1,21,22,24-28].

Industrial scale manufacture of albumin is generally based on integrated hybrid processes involving initial ethanol fractionation step followed by chromatographic steps like ion exchange chromatography and size exclusion chromatography which are used either to remove protein contaminants, or to separate out aggregates and accordingly to improve purity [29,30].

According to existing studies, implementation of different chromatographic methods, including IEX, SEC and affinity chromatography, in the plasma fractionation industry, may also target the removal of protein contaminants and aggregates from immunoglobulin preparations [1,31-34].

A number of studies can also be found in the literature review, in which different chromatographic methods have been used in the purification and manufacture of Von Willebrand Factor [15,20,38,39], F VII [40], F XI [41], F XIII [42], fibrinogen [43], thrombin [44,45], Protein C [46,47], Alpha 1-antitrypsin [48-50], and Antithrombin III concentrate [51-53].

Therefore, chromatographic procedures may be considered as essential purification tools in the large scale production of PDMPs.

Removal of plasma-borne viruses

One of the challenges in producing PDMPs is the possibility of plasma-borne viruses' transmission, due to the human origin of the starting material. Therefore, the implementation of specific virus inactivation or virus removal methods is necessary throughout the production chain of fractionated plasma products [1,54,55].

Recently, studies have revealed the usefulness of chromatographic purification in adding safety to PDMPs, by contributing to virus removal during manufacturing processes [1,2,10,56]. Howbeit, chromatography should only be considered as an ancillary means for viral safety.

Different chromatographic methods such as IEX and immunoaffinity chromatography, have been shown to potentially remove viruses with several physicochemical and biochemical properties [25,57,59].

For example, significant and efficient virus removals over chromatographic steps in FVIII manufacturing processes have been demonstrated in several studies [14,59,60].

Regarding F IX concentrate, introduction of chromatographic methods to the manufacturing processes has also led to virus partitioning [26,61,62].

Besides, chromatography plays a significant role in the viral safety of IgG preparations [58,63].

There are also some articles that Point out to the role of chromatography in virus removal of other PDMPs like albumin, ATIII, Alpha 1-antitrypsin and FVII concentrate [52,58,64].

Therefore, the use of chromatographic methods, alone or in combination, in the manufacturing processes of PDMPs, may improve their viral safety.

Removal of unwanted additives

Different types of chromatography may be used in plasma fractionation industry to remove virucidal agents, stabilizers, salts and other unwanted additives. PDMPs' Potential in transmission of plasma-borne viruses resulted in the introduction of specific virus inactivation or virus removal steps in their manufacturing processes [65,66].

The solvent-detergent treatment is a validated virus inactivation method in the manufacturing processes of PDMPs [1,67,68]. Following this step, the S/D agents have to be removed by additional downstream techniques like Chromatographic procedures [14,69-72].

Pasteurization is another validated viral inactivation procedure which requires Stabilizers to limit loss of protein functionality. However, since added stabilizers may decrease the rate and extent of viral inactivation, chromatographic methods may be used to remove them [53,73].

Chromatographic procedures may remove other unwanted additives such as salts too [74].

Quality control

Different chromatographic methods may be used in the qualitative and quantitative analysis of PDMPs with particular value in identification, impurity detection, lot release and stability studies [7,8,11,75-77].

According to existing guidelines, PDMPs must comply with the appropriate monographs of pharmacopeias. For example determination of molecular size distribution in human albumin solution and human normal immunoglobulin, by Size exclusion chromatography and measurement of citrate concentration in pooled human plasma by liquid chromatography are some of items cited in related monographs of European Pharmacopeia [78-81].

Conclusion

It can be concluded that the implementation and combination of selective and specific chromatographic procedures, play a crucial role in the large scale production of PDMPs for improving the quality, safety, purity, efficiency, yields and diversity of products.

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