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Quality by Design for peptide nanofiltration: Fundamental understanding and process selection

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AUTHOR-HIGHLIGHTS

- Nanofiltration is applied to the downstream process of a model peptide.
- Membrane transport is studied by Design of Experiments.
- Statistical models for solvent flux, peptide and salt rejections are obtained.
- Statistical transport models support dynamic process modelling.

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ABSTRACT

Recently, nanofiltration techniques have been introduced by pharmaceutical industries in the downstream processes for peptides, to perform concentration, purification and salt/solvent exchange, and have been demonstrated as being suitable for integration with conventional chromatographic purification techniques, providing savings in terms of time and costs. Design of Experiments (DoE) methods have been extensively applied in the process design of the conventional techniques, under the so-called Quality by Design (QbD) concept, but applications of DoE methods to nanofiltration are still few. In this study, the nanofiltration of a model peptide, conventionally named PEP_1, in trifluoroacetic acid/acetonitrile/water mixtures is studied by DoE. Statistical models for solvent flux, peptide and trifluoroacetic acid rejections are obtained by statistical Analysis of Variance and the best operating conditions for concentration are found by numerical optimization of the statistical models. The statistical models from DoE are included in the mathematical framework of the diafiltration process, to calculate the evolution of peptide, counter-ion and solvent concentrations over time, compare constant volume vs. variable volume diafiltration modes, and select the best process in terms of operating time and solvent consumption. This work demonstrates that empirical DoE models can provide phenomenological understanding of the transport mechanism through nanofiltration membranes for a specific solute of interest and successfully support the process modelling for concentration and diafiltration, providing a methodology to select the most appropriate filtration technique for a given separation problem.

1. Introduction

Recently, nanofiltration (NF) techniques have been introduced by the pharmaceutical industries as part of the downstream processes (DSP) for peptides, to perform concentration, purification and salt/solvent exchange, and they have been demonstrated as being suitable for integration with conventional purification techniques, providing savings in terms of time and costs (Marchetti et al., 2013). Application of NF has been supported by the development of new membrane materials with high stability in organic solvents (commonly used in the peptide industry), and efficient membrane modules (Tsuru et al., 2003; Yang et al., 2001; Vandezande et al., 2008).

During process development, process parameters and quality attributes are investigated, with the aim of establishing a relationship among them. Critical Quality Attributes (CQAs) are physical or chemical characteristics, which must be controlled to ensure the quality of the product. Critical Process Parameters (CPPs) are process inputs, which have a direct and significant effect on CQAs, when they are varied within the experimental range (Yu, 2007). Typical CQAs for a mixture after NF are specifications regarding concentration of main product, impurities, ionic and solvent composition. To support new initiatives and provide guidance for pharmaceutical

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process development, the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use introduced the Quality by Design (QbD) concept. This concept was defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, August 2009), and it has become an important tool in assisting the industry to move towards a more scientific approach to pharmaceutical development. According to the conventional framework for drug development, or Quality by Testing (QbT), little or no emphasis is placed on how the design of efficient and effective processes can ensure product quality. Product specifications are set by observing data from tests believed to be acceptable and expecting future products to have the same characteristics. Finished drug products are tested by assessing whether they meet the manufacturer's specifications. If they do not meet the requirements, they are discarded, without deep investigation of root causes for failure. Under QbD, consistency comes from the design and control of the process. QbD identifies characteristics which are critical to quality, translates them in attributes that the product should have, and establishes how the process parameters can be varied to produce a product with the desired characteristics. Effort is made to understand all the possible effects of CPPs on the final CQAs, and to identify all the possible sources of variability. As a result of all this knowledge, the company can (i) select the best process conditions with a limited number of experimental data; (ii) continually monitor the process to assure consistent product quality; and (iii) update the process without requiring further experimental effort.

Understanding and, possibly, modelling all the possible effects and sources of variations on the quality of the final product is often difficult. Consequently, Design of Experiments (DoE) methods are extensively applied in process design to help engineers understand the effects of possible combinations and interactions of various parameters on final drug quality (Shivhare and McCreath, 2010). Application of DoE provides scientific understanding of the effects of multiple process parameters on product CQAs and leads to the establishment of a "design space" and a manufacturing control strategy. Within QbD, design space is defined as "the multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide quality assurance" (Yu, 2007). DoE methods are much more efficient than the classical "one-factor-at-a-time" approach. The traditional approach demands considerable material expense and is more time consuming, since, to determine the effect of each factor, experiments may be designed to investigate one factor at a time so that all other independent variables (factors) are held constant. DoE, differently, is characterized by reduction or minimization of the total number of trials by the simultaneous variation of all potential influencing factors. The results analysis is characterized by information compactness, allows the identification of interactions among process parameters (impossible for the 1-factor-at-a-time approach), favours the selection of optimal working conditions and permits the determination of the design space of CPPs, based on acceptable range of CQAs.

DoE methods have been successfully employed for the development of chromatographic techniques, to identify the important factors affecting the retention performance and optimize the separation (Hibbert, 2012; Atkinson and Tobias, 2008). Studies of NF by DoE are, on the other hand, few. Examples of the application of DoE techniques to NF have been reported by Marchetti et al. (2013) for applications to peptide processes in acetonitrile (ACN)/water mixtures. Solute concentration, salt concentration, pressure and solvent composition were ascribed to influence the filtration performances (i.e. solvent flux, peptide and salt rejections) and the effects were discussed from a phenomenological point of view. Furthermore, Ahmad et al. (2009) investigated the separation of dye/salt/water solutions by porous ceramic membranes by DoE, and temperature, feed concentration, pressure and pH, examined by Response Surface Methods, were found to statistically affect the quality of the separation. The polynomial equations for the responses, developed by DoE, can be used to formulate objective functions for numerical optimization, in order to find the best operating conditions for achieving the separation of interest, as shown by Marchetti et al. (2013) for the nanofiltration of peptide solutions or by Polom and Szaniawska (2006) for the nanofiltration of acid lactic solutions. Finally, only a few studies show how information from DoE can be used to support process modelling for process selection. One good example is the work by Román et al. (2012), in which concentration-dependent solute rejection obtained by experimental design was used to describe the dynamics of different membrane filtration processes and select the most appropriate filtration technique for the demineralization of acid whey.

In this work, DoE analysis is used to investigate the NF performance of a model peptide, PEP_1, in ACN/water mixtures, with the dual purpose of characterization of membrane transport mechanism for this specific model peptide and process selection for the filtration step. Statistical models for solvent flux, peptide and trifluoroacetic acid rejections were obtained by statistical Analysis of Variance (ANOVA) and the best operating conditions for concentration were found by numerical optimization of the statistical models. The statistical models from DoE were included in the mathematical framework of the diafiltration process, to calculate the evolution of peptide, counter-ion and solvent concentrations over time, compare constant volume vs. variable volume diafiltration modes, and select the best process in terms of operating time and solvent consumption. This work demonstrates that empirical DoE models can provide phenomenological understanding of the transport mechanism through nanofiltration membranes for a specific solute of interest, and successfully support the process modelling for concentration and diafiltration, providing a methodology to select the most appropriate filtration technique for a given separation problem.

2. Materials and methods

2.1. Materials

A peptide produced by Lonza AG (Switzerland), and named PEP_1 in this work, was used as a case study. It has a molecular weight of 3000 g mol^{-1}, an isoelectric point of 4, and high water solubility (> 10 g l^{-1}). Nanofiltration (NF) is used to perform concentration, salt and solvent exchange for the model peptide in TFA-H/ACN/water mixtures, as part of its downstream process (cf. Fig. 1).

The peptide mixture exits the preparative chromatography with a composition of 0.06–0.1%v TFA-H/30%v ACN/water; concentration and diafiltration are applied to increase the peptide concentration, reduce the operating volumes and reach the composition of 0.02%v TFA/0.003%v ACN/water before entering the lyophilization. The

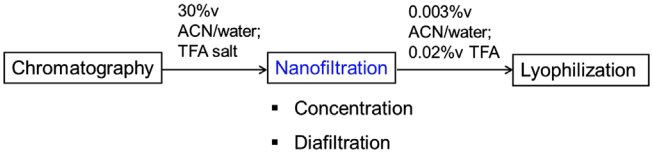

Fig. 1. Downstream process (DSP) for PEP_1.