Purification technologies for biopharmaceuticals

Researchers from CICECO – Aveiro Institute of Materials, Chemistry Department, University of Aveiro – in Portugal are working on the development of novel, cost-effective platforms for the purification of antibodies

Human ageing is continuously advancing and will become one of the key driving forces of societal change in the decades ahead. Specific needs of new affordable biopharmaceuticals to treat age and prosperity-related diseases will considerably increase, reinforcing the demand for scalable and cost-effective production technologies. Biopharmaceuticals have largely improved the treatment of many diseases and in some cases are the only approved therapies available for specific human disorders. These biologic-based products, which include antibodies, recombinant therapeutic proteins and nucleic acid-based products for *in vivo* medical purposes, find application in several medicinal areas, such as in vaccination, immunisation, oncology, autoimmune, cardiovascular, inflammatory and neurological diseases.

The biopharmaceuticals market, estimated at USD 199.7bn (~€179.3bn) worldwide in 2013, has been projected to reach $497.9bn by 2020.[1] Antibodies are amongst the most costly therapeutic options available on the market, and with the increasing competition between healthcare manufacturers, the success of the next generation of therapeutic drugs mainly depends on economic issues. In 2013, 970 biopharmaceuticals were under development, of which 338 were monoclonal antibodies (mAbs), 93 were recombinant proteins, 46 were gene therapy products and 15 were RNA antisense therapeutics.[2] However, and in addition to the large research investment on these therapeutics, antibodies present in hen egg yolk, namely immunoglobulin Y (IgY), are an alternative option that can be obtained in higher titres and at lower cost. As a class of antibodies, IgY can be also used to treat a wide variety of human disorders.[3]

Although less attention has been given to IgY, this large amount of alternative antibodies opens the door for a new class of cheaper biopharmaceuticals. Nonetheless, less than 2% of the total polyclonal antibodies produced worldwide are IgY.[4] This low percentage is mainly related to difficulties in isolating antibodies from egg yolk, which is essentially composed of a considerable lipid fraction dispersed in a water-soluble medium containing immunoglobulins. As a result, the production cost of high-quality/high-purity IgY still remains higher than other drug therapies due to the absence of a cost-effective purification strategy – the current cost of high-purity IgY reaches values up to €5.6m/g.[5] Trying to overcome their high cost, which currently restricts the widespread application of biopharmaceuticals as alternative therapeutics, Dr Mara G Freire, a co-ordinator researcher at CICECO – Aveiro Institute of Materials, Chemistry Department, University of Aveiro – in Portugal, has received funding from a starting grant from the European Research Council (ERC, IgYPurTech, ERC-2013-StG-337753) to develop cost-effective and sustainable purification techniques for antibodies. One of the distinctive features of this project relies on the use of ionic liquids (ILs) which can be tailored to meet the requirements of target applications. ILs are salts with low melting temperatures, and most of them are liquid at room temperature because they are usually composed of a large asymmetric organic cation and either an organic or inorganic anion. In addition to the ILs' negligible volatility and non-flammability, which have contributed to their common epithet attribution of 'green solvents', one of the main advantages of ILs relies on the possibility of tailoring their properties by an adequate rearrangement of their cation/anion combinations – 'designer solvents'. ILs can be designed to interact selectively with different types of solutes/solvents and improved properties and target applications can always be foreseen.

The research team

The research team supervised by Freire has multidisciplinary skills, with PhD and MSc students and post-doctoral fellows, with education in chemistry, chemical engineering, environmental engineering, biochemistry, biotechnology, health sciences and biology. Freire has been working with ionic liquids and on the development of alternative purification and concentration platforms for the last ten years as well as on the use of ILs for the extraction of compounds with therapeutic properties from biomass. In particular, ionic liquid-based aqueous biphasic

systems have been investigated for the extraction, concentration and purification of alkaloids, antioxidants, endocrine disruptors and pharmaceuticals, among others. Freire also has a physicochemical background on their thermophysical characterisation, on the determination of their liquid-liquid phase diagrams, and on the understanding of the molecular-level mechanisms responsible for phase transitions. Freire and her co-workers have developed novel concentration technologies to allow the identification and quantification of persistent pollutants in water streams, novel concentration methods to improve the detection of drugs in human urine, one-step concentration and purification techniques for the detection of cancer biomarkers in human fluids envisaging early-stage diagnoses, among others. Most of these achievements were obtained with systems composed of ILs due to the possibility of tailoring their properties and selectivity aiming at extracting a target biomolecule.[6] More recently, large efforts are being focused on the synthesis and use of natural-derived ionic liquids.

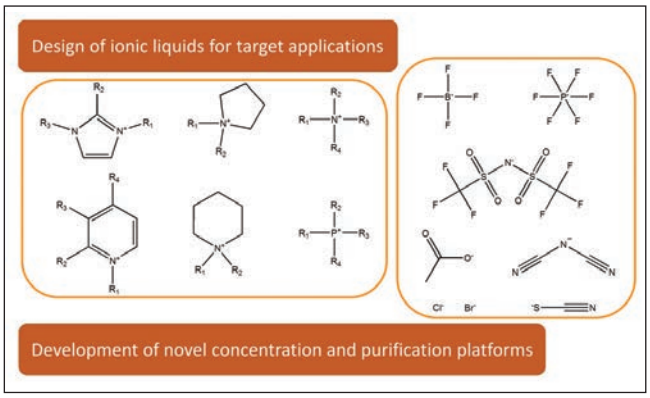
Current project outcomes

Although an ambitious project, the opportunity of an ERC starting grant and the creation of a research team, which is 100% committed to the development of new technologies for the purification of antibodies, allowed remarkable results to be obtained. Indeed, major efforts have been placed in these research activities, but, if it was easy, it would not be so exciting. Hitherto, three cost-effective technologies which lead to high-quality/high-purity IgY have been developed, and there is hope that their future application will permit the envisaged widespread use of biopharmaceuticals. At this point we are looking for potential collaborations or interest from industry to use/apply these technologies.

Given the remarkable results obtained with IgY, additional collaborations have already been arranged, including the establishment of a Portuguese consortium with researchers who carry out scientific activities on the biopharmaceuticals purification field. The creation of research consortiums with mutual goals allows the establishment of highly competitive networks by combining each partner's scientific and infrastructure strengths in order to generate new knowledge and capabilities in the biopharmaceuticals arena. Currently, Freire and co-workers are investigating the development of new technologies for the purification of other biopharmaceuticals, e.g. mAbs, recombinant proteins and nucleic acids, and where promising results are starting to appear.

Future prospects

The production and approval of biopharmaceuticals by regulatory authorities request effective and high quality biopharmaceuticals, i.e. with no side-effects, with a high degree of purity and preserved biological activity, which can only be attained through in-depth knowledge of the products and of their manufacturing processes. Our current experience of the development of new technologies for the purification of value-added



biopharmaceuticals paves the way for additional collaborations with other research groups and industrial companies in the frame of Horizon 2020. Europe has been at the edge of the production of biopharmaceuticals, and the outcomes of collaborative research activities represent a major contribution to maintaining European leadership in this field and, more importantly, to fighting the diseases characteristic of an ageing society.

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