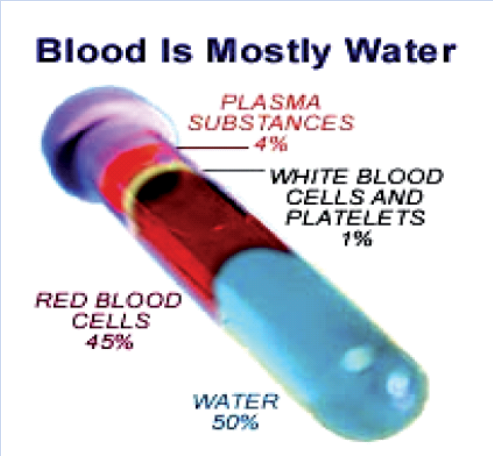
NovaSheet

Technology: AF4 - UV/Vis
Application: Poly-Hemoglobin for Blood Substitutes

Analysis Conditions / System Set-up

Sample:	Poly-Hemoglobin for Blood Substitutes
Solvent/Eluent:	0.1 M PBS Buffer, pH 7.4
Channel Flow:	1.50 mL/min
Cross Flow:	7.5 –1.5 mL/min in 1500 s
Separation System:	Postnova AF1000 Series Asymmetric Flow Field-Flow Fractionation
Detector 1:	Postnova PN3240 UV/Vis 4-Channel UV/Vis Detector (Wavelength 210 nm)

Polymeric Hemoglobin is one of the most interesting compounds in the area of synthetic blood research. World-wide research for blood substitutes is an ongoing task to overcome the limited resources of natural blood supplied by humans. The demand for blood is expanding more and more as a direct result of the growing age of the human populations and the ever increasing level of medical services supplied to the patients. Most of the complicated high tech surgeries (heart, cancer, etc.) require immense amounts of blood during the medical treatment.



For almost a hundred years saline has been used to replace lost blood volume. But it does not have the same electrolyte composition as human blood plasma and it is far less effective or safe when directly compared to blood plasma. Later this was then improved to Ringer's lactate, but saline is still being used for volume replacement.

At present, the most commonly used product to replace blood loss is the Ringer lactate solution; this is nothing more than a solution of electrolytes and glucose in concentrations similar to those found in plasma. But, it contains no plasma protein and no blood cells, so in more severe blood loss it is not enough to supply oxygen to the tissue.

To increase the oxygen-carrying capacity of the solution, red blood cell substitutes have to be added. These products fall into two categories, those solutions derived from hemoglobin, a natural human compound that carries oxygen, and the perfluorocarbons, which are synthetic compounds made from carbon that are able to carry oxygen.

The Perfluorocarbons under study have the advantage that they can be excreted through the kidneys and vaporized through the lungs. On the downside, they alter the way the body fights infections, producing a flu-like syndrome. Perfluoro chemicals are cheap and are completely free of biological materials so there is no risk of infectious agents contaminating them. In order to work, they must be combined with other materials that enable them to mix in with the bloodstream. These companion materials are fatty compounds known as lipids that take the form of an emulsion. They form a suspension of extremely small particles in a liquid that can be injected into a patient. The emulsion is relatively unstable and must be kept frozen until time of use.

The Poly-Hemoglobin solutions can be derived from outdated human blood, bovine sources, or synthesized by recombinant technology. The downside to hemoglobin solutions produced from outdated human blood is that there is still a potential risk that the material carries a disease. By using recombinant hemoglobin technology the disease risk can be eliminated. That is one reason most scientists focus on the recombinant hemoglobin.

But researchers are also facing another challenge. Every hemoglobin molecule in the human body is enclosed by a protective membrane or covering of the red blood cell. Once a hemoglobin molecule is removed from that covering, the hemoglobin tends to break apart into particles called dimers. These dimer particles are toxic and can cause severe damage to the lungs or kidneys. Thus many groups use glutaraldehyde to bind and polymerize the hemoglobin molecules together, so they don't break apart in the bloodstream.

In order to completely characterize these polymers, Asymmetric Flow FFF is the analytical tool of choice. In the following an example for the characterization of poly-hemoglobin using AF4-UV is shown.

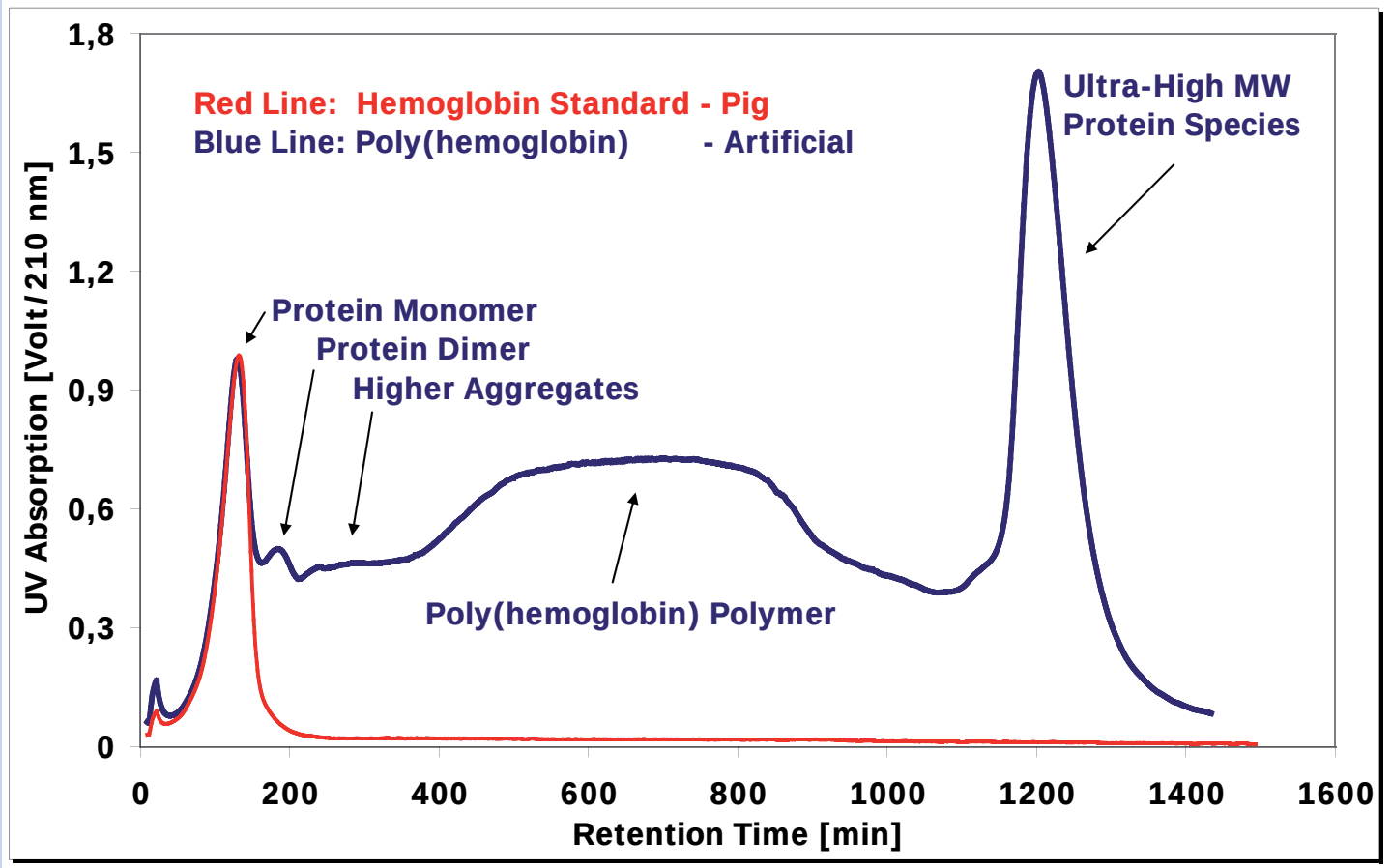


Fig.1: Characterization of Poly(Hemoglobin) using AF4 coupled with UV.

Why use Asymmetric Flow FFF and UV for the characterization of poly-hemoglobin?

- High resolution separation of protein & aggregates
- Fast, gentle and nearly interaction free separation without stationary phase.
- Fraction collection and easy direct coupling with further analytical techniques as MALDI, ELISA, etc.