Experimental report

21/10/2022

Proposal: 9-13-947 **Council:** 4/2020

Title: Hydration associated with drug release in albumin-based non-covalent drug delivery systems

Research area: Other...

This proposal is a resubmission of 8-05-456

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Samples: Human Serum Albumin
PEG 400
PEG 200
PEG 600
Deuterated PEG400
warfarin
Evans blue dye

Instrument	Requested days	Allocated days	From	To
D22	3	3	11/06/2021	14/06/2021

Abstract:

The natural transport function, multiple ligand binding sites, and cellular interactions provide a rationale for the exploitation of albumin for drug delivery. But, at the same time, albumin ability to bind between 5000 to 43000 of water molecules, to help regulate the blood pressure, poses the question of the role of water displacement during ligand/drug binding and release to and from albumin.
The present proposal aims to elucidate how changes in albumin hydration affect two model drugs (the highly specific warfarin and the non-specific Evans blue dye) release. Here, we focus on an osmotic stress method using small solutes (PEG200, 400, and 600) to affect albumin's structure and binding/release capacity.
We will also utilize the state-of-the-art 'NUrF' set-up comprising a continuous flow-through cell, enabling a wide range of mixture compositions to be delivered by automatic mixing using an HPLC pump; combined with in situ UV/Vis absorption and fluorescence spectroscopies, and an inline density meter. The enhanced information will provide new structural insights to drive the design of drugs with improved binding and release profiles.

Experimental report for proposal: 9-13-947

Hydration associated with drug release in albumin-based non-covalent drug delivery systems

Cedric Dicko, Judith Houston, Andrea Scotti, Adrian Rennie

Summary
The natural transport function, multiple ligand binding sites, and cellular interactions provide a rationale for the exploitation of albumin for drug delivery. But, at the same time, albumin ability to bind between 5000 to 43000 of water molecules, to help regulate the blood pressure, poses the question of the role of water displacement during ligand/drug binding and release to and from albumin.
The present proposal aimed to elucidate how changes in albumin hydration affect two model drugs (the highly specific warfarin and the non-specific Evans blue dye) release. Here, we focused on an osmotic stress method using small solutes (PEG200, 400, and 600) to affect albumin's structure and binding/release capacity.
We utilized the state-of-the-art "NUrF" set-up comprising a continuous flow-through cell, enabling a wide range of mixture compositions to be delivered by automatic mixing using an HPLC pump; combined with *in situ* UV/Vis absorption and fluorescence spectroscopies, and an inline density meter.

Results

The set-up

The setup comprises two main parts: (i) the flow handling system, and (ii) the measurement system (see figure 1 and 2).

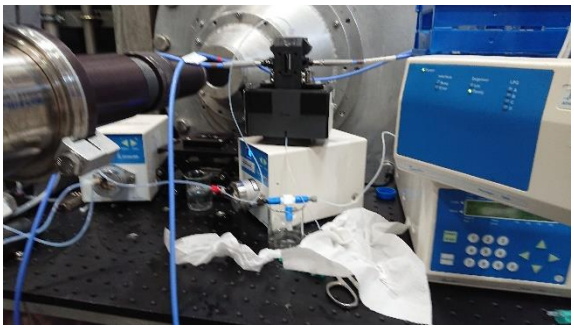

Figure 1. Setup with pumps and NUrF cell (black open box in the middle)

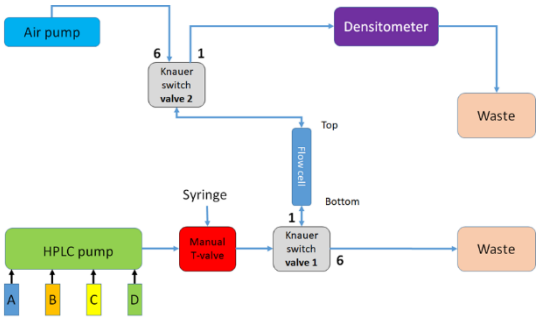

Figure 2. Flow handling diagram. The HPLC pump mixes and delivers the sample as well as washing fluids for the flow cell. A set of valves allow the sample or rinse to be measured or flushed. Finally, a dry air line, dries the flow cell.

The first challenge with setting up the experiment was the integration of the data collection (SANS, UV, Fluorescence and density) flow into Nomad. The second challenge was to handle and integrate the fluid in and out of the measuring cuvette.
Both integrations were successful, except for some issues with reading the density values and controlling the fluorescence light source.

The data

The planned experiments consisted of BSA + Warfarin drug + dPEG in D_2O. In the HPLC reservoirs (A, B, C and D) we had the following stock solutions: (A) 5mg/mL albumin in D2O, (B) 5mg/ml albumin + 30% w/w PEG (200, 400, 600). Or, (A) 5mg/mL albumin in D2O + drug, (B) 5mg/ml albumin + 30% w/w PEG (200, 400, 600) + drug.

In reservoir C and D, we had water and 20% ethanol for the cleaning and rinsing after each sample mix.

The four starting stock solutions are connected to the four channels of the chromatography system (A, B, C, and D). For each titration, a volume of the albumin in D2O is mixed with a volume of (albumin/PEG) in the following volume ratios: 100/0, 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80, 10/90, 0/100.

Figures 3, 4 and 5 summarises the change in Rg as a function of increasing PEG concentration.

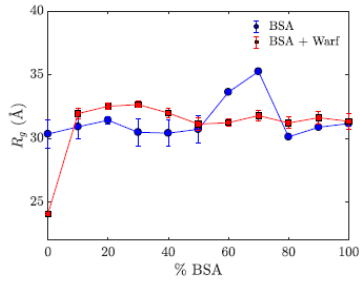

Figure 3. Change in Rg as a function of %BSA/dPEG200. Comparison of BSA and BSA + warfarin drug

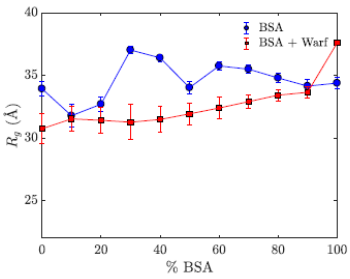

Figure 4. Change in Rg as a function of %BSA/dPEG400. Comparison of BSA and BSA + warfarin drug

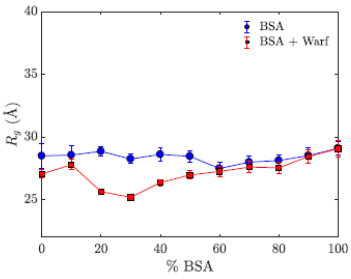

Figure 5. Change in Rg as a function of %BSA/dPEG600. Comparison of BSA and BSA + warfarin drug

Figure 6 illustrate the change in UV-visible absorbance of BSA in the presence of increasing dPEG 200. The second peak from the left, centered at 280nm is proportional to the concentration of BSA in the cuvette. Surprisingly, the peak was varying. The changes suggested that the BSA was not properly delivered to the flow cell.

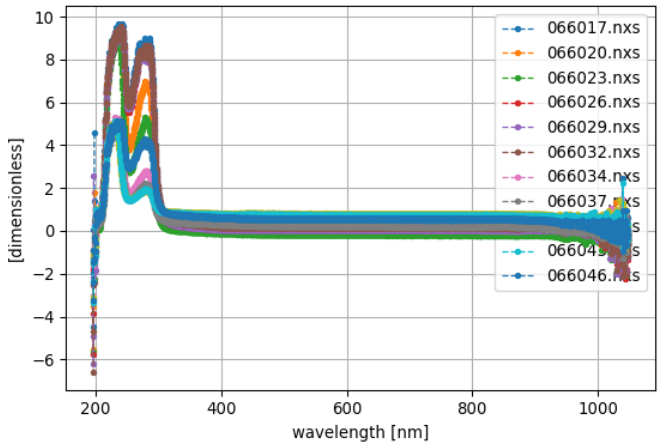

Figure 6. UV-absorption spectra of BSA in the presence of increasing dPEG200

Our efforts are focused on utilizing the concentration information from the UV to correct the SANS data. Most importantly, to also use the density data to estimate the real PEG concentration.
Unfortunately, the communication between the densitometer and Nomad failed. We have only a video recording of the densitometer screen over the beamtime period. Image analysis is needed to automatically read the density from the video.

Figure 7 illustrate the fluorescence emission spectrum of BSA in increasing dPEG200 concentration.

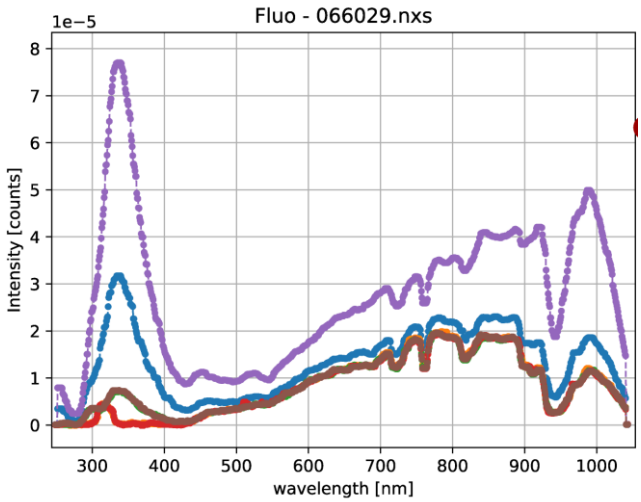

Figure 7. Fluorescence emission spectrum of BSA in increasing dPEG200 concentration. With increasing PEG the main fluorescence peak intensity (left) decreases.

Unfortunately, the miscommunication between Nomad and the fluorescence monochromator (to set the excitation wavelength) and the fluorescence spectrometer were difficult. As a results most fluorescence experimental results are incomplete.

Conclusion

Overall, we have demonstrated the integration of the NURF and liquid flow handling in Nomad to achieve automated titration experiment. A few communication issues resulted in incomplete data sets.

The results showed that UV as a diagnostic (concentration) was invaluable to understand that BSA concentration was fluctuating and hence influencing the SANS. We expected similar insights from the densitometer regarding the PEG concentration.

Although early, it appears that changes are most significant in fluorescence. There we are mostly sensitive to the BSA tertiary structure and solvent interaction.