Rapid contrast matching by microfluidic SANS

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We report a microfluidic approach to perform small angle neutron scattering (SANS) measurements of contrast variation and matching, extensively employed in soft and biological matter research. We integrate a low scattering background microfluidic mixer and serpentine channel in a SANS beamline to yield a single phase, continuous flow, reconfigurable liquid cell. By contrast with conventional, sequential measurements of discrete (typically 4-6) solutions of varying isotopic solvent composition, our approach continually varies solution composition during SANS acquisition. We experimentally and computationally determine the effects of flow dispersion and neutron beam overillumination of microchannels in terms of the composition resolution and precision. The approach is demonstrated with model systems: H\textsubscript{2}O/D\textsubscript{2}O mixtures, a surfactant (sodium dodecyl sulfate, SDS), a triblock copolymer (pluronic F127), and silica nanoparticles (Ludox) in isotopic aqueous mixtures. The system is able to zoom into a composition window to refine contrast matching conditions, and robustly resolve solute structure and form factors by simultaneous fitting of scattering data with continuously varying contrast. We conclude by benchmarking our microflow-SANS with the discrete approach, in terms of volume required, composition resolution and (preparation and measurement) time required, proposing a leap forward in equilibrium, liquid solution phase mapping and contrast variation by SANS.

1 Introduction

Small angle neutron scattering (SANS) is a powerful, highly penetrating and non-destructive probe of the molecular to nanoscale structure and interactions of matter, at typical length-scales of \( \sim 1 \) nm to approximately 500 nm.\textsuperscript{1-3} The approach is complementary to a range of microscopic, spectroscopic, rheological and notably scattering approaches, but derives its contrast from nuclear interactions which can vary significantly between isotopes and between elements of low atomic weight. SANS is thus extensively used in the study of complex and biological fluids, including systems containing surfactants, nanoparticles or colloids, polymers and proteins, and their spontaneous or directed-assembly.\textsuperscript{3-5}

SANS experiments generally require high-flux neutron beams (\( \sim 10^{6}-10^{8} \) neutrons \( \text{cm}^{-2}\text{s}^{-1} \)) and are therefore carried out at large scale facilities – nuclear reactors or pulsed sources – whose access is competitive and generally restricted to periods of the order of 24h to several days. Conventional liquid-phase SANS measurements involve the sequential exposure of arrays of (approximately 10-40) samples containing individually-prepared, often multicomponent solutions. In order to reduce scattering background, sample cells are generally manufactured in amorphous quartz or fused silica and, since beam footprints have dimensions of \( \sim 1 \) cm\(^2\), these are relatively large cuvettes of pathlengths of 0.5-2 mm. The actual SANS measurement time per sample varies significantly with solute concentration, scattering contrast and measurement statistics required, but typically ranges from 1 s to 2 h. Sample preparation, cell loading and cleaning thus require an amount of time and effort comparable or larger than measurement times, resulting in downtime and thus inefficient use of scarce neutron beamtime. More importantly, the precision of thermodynamic phase mapping and contrast variation (CV) becomes restricted by the finite number of measurements feasible, and often requires several iterations to establish precise phase boundaries of multicomponent systems, or sizes and shapes of molecular assemblies in solution. The significance of CV stems from its ability to elucidate the structure of individual species or components in multicomponent systems. For instance, in order to study a core-shell colloid, by varying the H/D ratio of the solvent, the scattering intensity can made proportional to the particle’s core (i.e. when the shell is contrast-matched), the polymer shell...
or both. CV also plays a significant role in resolving the structure of highly concentrated systems, where inter-particle correlations dominate the scattering signal.

In this paper, we seek to couple microfluidics and SANS to resolve this (i) sample preparation bottleneck, as well as (ii) improve compositional precision, (iii) increase mapping speed and (iv) measurement versatility, by developing a configurable SANS liquid cell based on a microfluidic platform.

Microfluidics is routinely coupled with an array of characterisation approaches, including dynamic light scattering and small angle X-rays scattering (SAXS). Microdevices accurately formulate mixtures, requiring minute sample volumes and manipulate flow fields with unprecedented precision. Automated sample preparation microfluidic platforms for SAXS of biological systems have been previously demonstrated, based on either sequential mixing, stopped-flow SAXS and sample wash, or by multi-sample, parallel formulation and measurement, greatly improving measurement throughput. The generation of spatio-temporal concentration gradients via diffusion has also been exploited to resolve kinetic processes.

Coupling with SANS is comparatively challenging due to typically large beam sizes (\(\sim\) mm-cm) with respect to channel dimensions (\(\sim\) 1-1000 \(\mu\)m), large sample volumes (\(\sim\) 0.5-1 mL) and/or long acquisition times required for SANS. We have recently demonstrated a microflow SANS processing study of concentrated surfactant lamellae, with beam sizes as small as 500 \(\mu\)m and single microchannels of \(\sim\)50 \(\mu\)m widths. A model lyotropic liquid crystal surfactant solution with well-defined, constant, composition was subjected to a variety of flow fields to determine their consequence at the molecular and nanoscales. However, at present, the feasibility of such single-channel studies is limited to strongly scattering systems, with characteristic scattering intensities of the order of, or in excess, of \(\sim\)100 cm\(^{-1}\), within second timescales. Single channel acquisition for systems of \(\sim\)1 cm\(^{-1}\) characteristic intensity requires approximately 3-5 min per spectrum, which is generally excessive for high-throughput scanning.

The current paper demonstrates microfluidic SANS of such ubiquitous weakly-scattering systems (below \(\sim\)1 cm\(^{-1}\)), including colloidal suspensions, surfactant and polymer mixtures at relatively low concentration, and resorts to the beam overillumination of several microchannels to attain the required scattering statistics. We carry out, arguably, the most common type of SANS measurement, with a large number of steps \(N\) \((\sim 10^4-10^5)\) of a solvent matrix, as function of the composition of hydrogenous and deuterated isotope, and selected analytes. Light and heavy water are shown for illustration. (b) Contrast factor \(\Delta \rho^2 = (\rho_{\text{solute}} - \rho_{\text{solvent}})^2\) as a function of H/D matrix concentration. Open circles correspond to the conventional, discrete approach, illustrated in (c), while dense grey points correspond to microflow-SANS method, shown in (d). (c) Conventionally, a series of \(N\) solutions are individually prepared and loaded onto cuvettes for measurement. By contrast, in (d) the fluid composition is adjusted continuously in microfluidics, following a specific stepped profile \(F_A(t)\) and \(F_B(t) = F_{\text{tot}}(t) \cdot F_A(t),\) with large number of steps \(N\) \((\sim 10^5)\).

Fig. 1 Comparison between conventional and microfluidic contrast matching in SANS. (a) Neutron scattering length density \(\rho\) of a solvent matrix, as function of the composition of hydrogenous and deuterated isotope, and selected analytes. Light and heavy water are shown for illustration. (b) Contrast factor \(\Delta \rho^2 = (\rho_{\text{solute}} - \rho_{\text{solvent}})^2\) as a function of H/D matrix concentration. Open circles correspond to the conventional, discrete approach, illustrated in (c), while dense grey points correspond to microflow-SANS method, shown in (d). (c) Conventionally, a series of \(N\) solutions are individually prepared and loaded onto cuvettes for measurement. By contrast, in (d) the fluid composition is adjusted continuously in microfluidics, following a specific stepped profile \(F_A(t)\) and \(F_B(t) = F_{\text{tot}}(t) \cdot F_A(t),\) with large number of steps \(N\) \((\sim 10^5)\).
and considerations of flow dispersion and residence time distribution. We then calibrate and validate the approach with ubiquitous water isotopic mixtures, and then demonstrate microflow-SANS with a common surfactant (sodium dodecyl sulfate, SDS), silica nanoparticles (Ludox), as well as a triblock copolymer (pluronic F127) in aqueous solution. We conclude with an outlook of this approach, benchmarking it against the conventional discrete methods and discussing its limitations.

2 Materials and methods

We next briefly introduce SANS contrast variation (CV) and contrast matching methods, followed by a description of the microfluidic-SANS CV approach, discussing key control parameters and optimisation.

2.1 Contrast variation

The differential scattering cross-section of a sample can be expressed as:

\[
\frac{d\Sigma(q)}{d\Omega} = \left(\frac{N}{V}\right) V_{p}^{2} \Delta \rho^{2} P(q) S(q) + B
\]

where \(N/V\) is the number density of the scattering object, \(V_{p}\) is the volume of the object (e.g. a colloid), \(\Delta \rho = \rho_{\text{solute}} - \rho_{\text{solvent}}\) the difference between the scattering length density of the solute and that of the solvent or medium \(\rho_{\text{solvent}}\), \(P(q)\) is the form factor, \(S(q)\) is the structure factor, and \(B\) is the 'background'. The (elastic) momentum transfer \(q \equiv (4\pi/\lambda)\sin(\theta/2)\), where \(\lambda [\AA]\) is the neutron wavelength (typically 5-15 Å) and \(\theta\) the scattering angle. In simple terms, \(P(q)\) characterises the size and shape of the scattering object, and \(S(q)\) the spatial arrangement and interactions between objects. While the first 'coherent' term of eq. 1 contains relevant structural information, term \(B\) is a \(q\)-independent term, a constant, accounting for the incoherent scattering signal of the sample (predominantly due to \(H\)) and for fluctuations with correlation lengths smaller than \(q^{-1}\). The scattering length density is defined as \(\rho = \sum_{i}^{n} b_{i}/V\), corresponding to the sum of scattering lengths \(b_{i}\) of relevant atoms within the volume containing \(n\) atoms. Since \(b_{i}\) has dimensions of length, \(\rho\) has dimensions of length \(^{-2}\). For convenience, often \(\rho = \sum_{m} b_{m} \rho_{m} N_{A}\), where \(\rho_{m}\) is the bulk mass density of the scattering object [\(\text{g cm}^{-3}\)], \(N_{A}\) is Avogadro’s number [\(\text{mol}^{-1}\)] and \(M_{w}\) is its molecular weight [\(\text{g mol}^{-1}\)], and all atomic \(b_{i}\) are tabulated.\(^{36,37}\)

Since \(\Delta \rho^{2}\) modulates the coherent scattering term, it is commonly referred to as the ‘contrast factor’.

In a contrast variation experiment, the contrast between the solute and its solvent \(\Delta \rho\) is systematically varied, in order to find the contrast match point, \(\Delta \rho = 0\), and thus determine exactly \(\rho_{\text{solute}}\) against a known \(\rho_{\text{solvent}}\), generally a solvent mixture. This is illustrated in Fig. 1a, where the solid white line indicates the \(\rho\) of mixtures of water and heavy water which, at different ratios, equal those of surfactant SDS or silica nanoparticle (Ludox). Figure 1b, depicts the parabolic \(\Delta \rho^{2}\) dependence on water composition, including the match point, where it vanishes.

In conventional discrete measurements, a number of samples \((N, \sim 5-10)\) is tediously prepared and loaded into cuvettes onto a sample changer for sequential measurement and analysis, as depicted in Fig. 1c. After calibration and background subtraction, these data can be converted in \(\Delta \rho^{2}\) (shown as circles in Fig. 1b) and fitted to yield the ‘match point’ with a given precision.

In this paper, we propose to automate the CV experiment by the means of a microfluidic approach, depicted in Fig. 1d. While the device has multiple inputs, we illustrate the concept with two input solutions A and B prepared at the same concentration of solute, but different isotopic compositions of the solvent (e.g. D\(_{2}\)O and H\(_{2}\)O), injected within a microdevice with a mixer and long serpentine channel. By continuously changing the input relative flow-rates \(F_{A}:F_{B}\) (while maintaining the overall rate, \(F \equiv F_{A} + F_{B}\), constant for simplicity), shown in 1e, a rapid scan of \(\Delta \rho\) is trivially achieved, yielding the grey line in 1b with exceptional and tunable precision. Evidently, both the accuracy and precision of the approach are predicated on a number of microfluidic control variables, diffusive mixing and dispersion, and SANS acquisition and statistics, discussed in detail in the next section.

2.2 Sample preparation

Isotopic mixtures of ultra-pure deionized water (Millipore) and deuterated water (D\(_{2}\)O, Sigma-Aldrich 151882), at various ratios, were prepared in volume terms \((v/v)\), and components weighted to increase composition accuracy. Sodium dodecyl sulfate (SDS, ≥ 99.0% purity, Sigma-Aldrich 436143) 10% weight by volume aqueous solutions were prepared and allowed to equilibrate for 24h. Stabilised silica nanoparticles with nominal diameter 12 nm, dispersed in 40% w/w in water (Ludox HS-40, Sigma-Aldrich 420816) were purchased and used as received. Ludox solutions were prepared at 5.2% w/v % concentration, by addition of H\(_{2}\)O or D\(_{2}\)O to the stock solution (in 1 part to 10 by volume), and adjusting it to pH 9 by addition of NaOH or NaOD, respectively. Solutions of triblock copolymer PEO-PPG-PEO pluronic F-127 (Sigma-Aldrich P2443, \(M_{w}\) ≈ 12.6 kgmol\(^{-1}\)) 2% w/v solutions were prepared in H\(_{2}\)O and D\(_{2}\)O. All solutions were stored at 25°C.

2.3 Microfluidic setup

The proposed microfluidic approach can evidently be implemented in a variety of neutron-compatible chips, including microdevices fabricated by frontal photopolymerisation (FPP), developed previously,\(^{29-31,38}\) or with commercially-available microchips with adequately low SANS background. These include microdevices fabricated in aluminium, fused silica or quartz, preferably with low thickness (\(~\text{mm}\)), but not, for instance polydimethyl siloxane (PDMS), as its scattering contribution is generally excessive and the microchannels deformable, thus compromising the absolute calibration of the SANS data. In this validation paper, we employ a commercial glass microreactor chip (Dolomite 3 port, 250 \(\mu\)l, 3000281) comprising three inputs, a double-T mixing junction, followed by a narrow mixing channel and a serpentine ‘reaction’ channel, with a rounded rectangular cross-section of 250 \(\mu\)m (height) \(\times\) 400 \(\mu\)m (width), and an overall channel length \(~\text{3041 mm}\). The device ‘glass’ is a high optical transmittance crown glass B270 from Schott, used
in biotech applications; conventional borosilicate glass is generally unsuitable due to its high boron (a strong neutron absorber) content. This device has thus a neutron transmission of \( \approx 91\% \), as detailed in Supplementary Information Section I. For all data presented in this paper, only two inputs were connected. The stock solutions were loaded into 10 mL plastic syringes (BD) and connected to the device by fluorinated ethylene propylene tubing (FEP WZ-06406-60 Cole-Parmer) with 0.8 mm internal diameter. The fluids were injected using Braintree Scientific BS-8000 syringe pumps, controlled via a custom-developed LabVIEW interface. For each SANS run, a total volume of 1-4 mL of (dilute) solution was employed, depending on statistics and composition resolution required. For all experiments presented in the main paper, we have fixed the total flow rate \( F \) at 0.1 mL min\(^{-1} \) (additional data series varying \( F \) are presented in Supplementary Information).

2.4 SANS data acquisition and analysis
The SANS experiments were carried out at the D22 diffractometer, Institut Laue Langevin (Grenoble, France). An incident neutron wavelength of \( \lambda = 6 \text{ Å} \), collimation of 5.6 m, and two sample-detector distances of 1.4 m and 5.6 m, were employed, yielding a wavenumber range of \( 0.009 \leq q \leq 0.61 \text{ Å}^{-1} \) (with offset detector) and \( 0.0027 \leq q \leq 0.042 \text{ Å}^{-1} \), respectively. For most datasets, a single sample-detector distance was measured, covering the relevant \( q \)-range for the system (e.g., a distance of 1.4 m was chosen for SDS and 5.6 m for Ludox solutions).

A square beam of 1 cm \( \times \) 1 cm footprint was employed, set by a cadmium diaphragm, thus overilluminating several microchannels. The microdevice was mounted onto a pedestal and attached to a xyz goniometer stage to enable the precise positioning of the microdevice with respect to the neutron beam. In all experiments, the device was placed such that the beam centre corresponded to a distance of 2670 mm from the double-T junction mixer. Employing a diffuse laser source, the illuminated sample area was found to cover 16 (full) and 2 (partial) microchannel widths, which was precisely determined for data calibration.

A semi-transparent beamstop enables simultaneous scattering and transmission measurements, which is advantageous for data calibration and avoids running identical experiments twice. The recorded data were corrected by empty cell and electronic background subtraction, and the intensity calibrated to absolute units [cm\(^{-1}\)] by the direct neutron beam flux. GRASP 7.15 was employed for data reduction, and IgorPro 6.37 and SASView 4.0.1 for data fitting.

When overilluminating microchannels, both the scattering signal and transmission contain contributions from the sample of interest and material background. This renders the data reduction somewhat complex since the microdevice cross-section varies spatially (unlike in usual ‘sandwich’ cell arrangements) and both the scattering and transmission of the sample contribution varies temporally. A detailed derivation and implementation of the data reduction is thus provided in Supplementary Information Section I. The dependence of SANS scattering of isotopic H\(_2\)O:D\(_2\)O mixtures on ratio and sample thickness (0.25-2 mm) is discussed in Supplementary Information Section II and Fig. S1.

3 Results and discussion
3.1 Microfluidic-SANS design of experiment

3.1.1 Ensuring (diffusive) mixing of inputs
The microdevice employed relies on diffusive mixing of inputs, and generally operates at a total flow rate \( (F) \) of 0.02 - 0.2 mL min\(^{-1}\), corresponding to a Reynolds number of \( Re \approx 10^{-2} \) to \( 10^{-3} \), in the laminar regime. The injection and lateral diffusion of co-flowing inputs is illustrated in Fig. 2a and, at steady state, transversal mixing is expected to occur at time \( t \) [s] when \( w_{mix} = \sqrt{2Dt} \) reaches the width \( w \) [m] of the channel, where \( D \) [m\(^2\)s\(^{-1}\)] is the diffusion coefficient of the species. From the flowrate \( F \), channel cross-section \( A \equiv w \times h \), the mixing width can be readily calculated at a given channel length \( L \) as \( w_{mix} = \sqrt{2DL/AF} \).

Since the solute concentration is constant in both inputs, we take \( D \) to be the self-diffusion of water, \( D_{H_2O} \approx 2.3 \times 10^{-9} \text{ m}^2\text{s}^{-1} \).
and estimate the mixing length for three typical flowrates (F_L = 0.02, F = 0.1 and F_H = 0.2 mL min\(^{-1}\)). The extent of mixing w/w_{mix}, under these conditions, is depicted in Fig 2b. At the highest flow rate F_H, the mixing length is estimated to be 1300 mm from the T-junction. For reference, the lateral diffusion of molecular and micellar SDS in water,\(^{40}\) is also indicated, with the latter increasing the mixing length up to 2650 mm at rate F.

### 3.1.2 Flow dispersion and residence time distribution (RTD)

Since the CV experiment relies on varying the ratio of inputs - and thus composition - over time, Taylor-Aris\(^{41–43}\) axial dispersion (i.e. along the direction of flow) must be kept to a minimum to ensure adequate composition resolution. From the velocity distribution and length of the channel, the probability distribution function describing the time one fluid element spends in a channel, is computed as a residence time distribution:\(^{44}\)

\[
RTD = \frac{L}{u} \sqrt{\frac{u^3}{4\pi D^2t}} - \frac{(L-w)^2}{4\pi D^2 \Delta t} \tag{2}
\]

where u [ms\(^{-1}\)] is the average velocity, D\(^*\) is the dispersion constant, defined as D\(^*\) = u\(^2\)d\(t\)/192D, where d\(t\) [m] is the hydraulic diameter (for a rectangle: 2wh/(w + h)) and L [m] is the distance travelled. Figure 2c shows the RTD width (estimated as \(\sqrt{2}\sigma\), where \(\sigma\) is the standard deviation of a fitted Gaussian) as a function of channel position L for the three representative flowrates (F_L = 0.02, F = 0.1 and F_H = 0.2 mL min\(^{-1}\)). Evidently, compositional dispersion increases with L and, based on these results, for this series of experiments we select to illuminate the device centred around L = 2670 mm. Following from Fig. 2b, this position ensures full (transversal) mixing of a range of systems, including isotopic water and up to (SDS) micellar solutions, at relevant flow rates, yet ensures that the RTD width remains relatively narrow to allow for compositional mapping. An optimisation of beam position can be made, however, for individual systems, placing the microdevice such the neutron beam illuminates a microchannel section at the earliest, fully mixed position L, shown in Fig. 2b, and therefore keeping the RTD width to a minimum.

### 3.1.3 Compositional average due to beam overillumination of microchannels

The SANS scattering intensity is directly proportional to sample volume and, since microchannel thickness h = 250 \(\mu\)m and width w = 400 \(\mu\)m are generally insufficient for weakly scattering systems (of characteristic intensity \(\sim\)1 cm\(^{-1}\)), we resort to overilluminating several microchannels. This has two significant consequences, of (i) rendering the data reduction more laborious, as discussed above, and (ii) averaging the SANS signal over solutions of varying composition across the illuminated area. Considering a typical circular beam of 12 mm diameter, 20 microchannels are thus illuminated, and the overall weighted RTD broadens further, as illustrated by the coloured lines in Fig. 2c and envelope RTD centred around L = 2670 mm.

Evidently, the shape of the beam footprint can also impact the overilluminated RTD. In case of a packed serpentine channel, in a rectangular arrangement, a square or rectangular beam footprint (instead of circular), with long axis along the primary channel direction will minimise compositional spread due to overillumination (detailed in Supplementary Information Section III and Fig. S2). For all experiments reported here, a square beam of 1 cm \(\times\) 1 cm was employed, such that the same configuration could be used for discrete measurements using ‘banjo’ cells of commensurate dimensions. In this microdevice, the area fraction containing sample is approximately 66% of the beam footprint and the effective, illuminate, sample volume is approximately 16.5 \(\mu\)l. As reference, in a standard 1 mm thick Hellma cell, this volume is 300 \(\mu\)l.

### 3.1.4 SANS acquisition time, statistics and composition resolution

Data statistics and composition resolution of the CV measurement series are jointly dictated by the (i) SANS acquisition time per spectrum (typically 5-20 s) and the (ii) total flow rate F, (iii) the number of composition steps and (iv) duration of the overall experiment. The latter two effectively define the rate of change of the flow rates F_A and F_B which sets a total number of points for a given experiment duration (e.g. 10 min).

Each scan comprises thus N discrete composition steps of \(\Delta t_\phi\) [s] duration. For convenience, we select the SANS acquisition time \(\Delta t_{SANS}\) to be equal to \(\Delta t_\phi\).

For the systems and concentrations investigated, a \(\Delta t_{SANS} \sim 5-20\) s is adequate, at the illuminated sample volume. The next step is to select a desired composition resolution for the \(\Delta \rho^2\) curve, in terms of the number of steps N. Under these conditions, evidently, increasing N increases (linearly) the total experimental time and volume required, as illustrated in Fig. 2d. Specifically, at \(\Delta t_\phi=\Delta t_{SANS}=10\) s, a CV curve with 12 points yields a nominal composition of resolution of 7.5 % (volume by volume composition) and overall experiment duration of 2 min. Increasing the total experiment time to 10 min, enables N=60 and thus a nominal resolution of 1.3 % v/v. In addition, axial dispersion and overillumination degrade this resolution, and can be estimated by comparing \(\sqrt{2}\sigma\) (RTD) with \(\Delta t\). From Fig. 2c, this value is approximately 8 s. Note however, that the distribution is centred on the correct composition, and this effect is thus generally small and can only be exactly quantified based on the impact of composition on the SANS response.

Further, axial dispersion can be reduced by increasing F and thus the Péclét number (Pe), at constant experimental time, as shown in Fig.2e, provided that transversal diffusive mixing is attained at a channel position L ahead of the measurement window. Furthermore, F sets the mean value of the RTD, but also the delay of the response of the system (i.e. the start-up time, and thus SANS measurement deadtime, which we seek to keep to a minimum, as discussed below). For comparison purposes, we have fixed measurement L to 2670 mm, which is compatible with all three flow rates F and D_{H,O} explored, but further refinement is possible on a system by system basis.
3.2 Microflow-SANS system validation with isotopic water mixtures

3.2.1 Step response

In order to validate the RTD estimations of eq.(2) and thus the compositional accuracy of the SANS measurements in the microdevice, the SANS response following a step change of the input concentrations was measured experimentally. The system was first filled at 95 % water (F_A) and 5 % heavy water (F_B) and allowed to stabilise for 3 min. A linear ramp function of N=120 steps, each one lasting Δt=5 s, was imposed until the symmetric condition was obtained as shown by the dashed line in Fig. 3d. The SANS acquisition time per spectrum was also fixed at Δt=SANS=5 s. The total duration of the experiment was 15 min, including 10 min for the ramp, and an additional 5 min wait time (exceeding the required 3 min response time). As above, the scattering intensity was recorded and averaged, for each frame, over the entire q range, and plotted in Fig. 3d in blue. For illustration I(q) curves extracted every 50 s are plotted in Fig. 3c to emphasise the fact that Δt=5 s yields considerable data scatter but clearly distinct averaged values.

The mapping of SANS data response and isotopic composition input establish the compositional accuracy of the microfluidic system and selected operating parameters, in both step and ramp responses, despite the low level of scattering of this solvent mixture. We next demonstrate rapid CV measurements with an array of model systems.

3.3 Contrast variation experiments

3.3.1 Silica nanoparticles in isotopic aqueous mixtures

The CV and matching of dispersed silica nanoparticles (Ludox HS-40) was demonstrated with N=120 and Δt=5s, requiring 10 min total experimental time. The device was first filled and allowed to stabilise for 3 min at 0.1 mL min⁻¹ (water stream F_A=0.95 F_sat), before ramping up the flow rate of D₂O-rich input, thus decreasing H₂O, as shown in Fig. 4b. Since the stock Ludox dispersion is suspended in H₂O (as detailed in the materials section), the solvent composition thus ranged from 5.2 to 87.4 % w/v D₂O.

The calibrated total scattering intensity, I(q), is plotted as a function of steps N in figure 4a. The minimum of the surface plot is rapidly identified as the contrast matching point. In order to determine the corresponding match composition, two methods can be employed. From the flow ramp response, including delay time and RTD, the composition at every N step can be calculated. However, in most cases, a direct experimental method is made possible by the decoupling of the background B and the coherent signal which is proportional to Δρ². Specifically, in order to precisely determine the solvent H₂O:D₂O composition, the I(q) curve was divided into two regions: a ‘low-q’ (from 0.01 to 0.053 Å⁻¹) and a ‘high-q’ (from 0.09 to 0.16 Å⁻¹), where incoherent scattering predominates. These q intervals are depicted in Fig. 4a, and the scattering data were averaged within these ranges. Term B is q-independent and linearly proportional to isotopic ra-
The rapid CV of common surfactant SDS, at a micellar composition of 10% w/v, was initially attempted with $N=120$ and $\Delta t=5\text{s}$, scanning a solvent composition space ranging from 10 to 90% v/v D$_2$O, shown in Fig. 5a, labelled ‘full-scan’. By contrast with the above, the challenge of this system is that the expected $\rho$ of SDS (0.33 $10^{-6}$Å$^{-2}$) is rather close to that of water. The measurement is thus susceptible to low composition resolution and relatively high incoherent scattering, around the match conditions. Prior to the CV ramp, the device was filled at $F=0.1$ mL min$^{-1}$ (water stream $F_A$ at 95% of $F_{tot}$) and allowed to stabilise for 1 min.

As above, the recorded total scattering $I(q)$ data were divided in a ‘low’ (<0.2 Å$^{-1}$) and ‘high’ (>0.4 Å$^{-1}$) $q$ range and averaged within. The ‘high’-$q$ data were predominantly incoherent and are plotted as black points in Fig. 5b. In order to extract averaged ‘coherent’ data, the ‘high’-$q$ was subtracted from the ‘low’-$q$
Fig. 5 Contrast variation microflow SANS for an 10% SDS in H₂O:D₂O solvent background. (a) Imposed ramp, at a fixed total flowrate of 0.1 mL min⁻¹. (b) Corrected scattering intensity (black) compared to the background (solvent) scattering intensity. (c) Zoom-in experiment, refining the data in (b) within 95:5 to 70:30 H₂O:D₂O range, enabling the precise location of the contrast match point. (d) Corrected scattering intensity from the full range (grey) and zoom-in (black) experiments. The magnified graph shows the contrast matching point of the solute.

and is plotted as grey points in Fig. 5b. The contrast match point is not discernible, but is clearly \( \leq 20\% \). We therefore perform a refinement scan by ‘zooming’ into the relevant composition space. We select a switch ramp from 6.5 % to 25 % v/v D₂O, with corresponding flow rate ratios indicated over Fig. 5b. For comparison, we maintain \( N=120 \) but increase \( \Delta t \) to 10s to further improve statistics. The flow rate profile is also depicted in Fig. 5a, and labelled 'zoom-in'. Following the same data treatment, a clear minimum in the coherent component is now identified, as shown by the grey dataset in Fig 5c, establishing the value of partial scans to effectively select the desired composition resolution for a CV measurement. The two coherent datasets, obtain for the coarse and fine scans, are superposed in Fig. 5d, confirming the agreement and reproducibility between measurements. Note that the coherent signal does not completely vanish at the match point, for instance in Fig. 5c, which is likely due to the micellar, headgroup-tail, structure and the match point corresponding the dominant 'tail' signal. An even higher resolution scan would be required to separately match the headgroup signal. Overall, the experimental data show a match point at 12.8 % v/v D₂O, corresponding to \( \rho=0.33\times 10^{-6} \text{ Å}^{-2} \), in line with previous reports.

Fig. 6 (d) 2D data showing the contrast variation experiment, decreasing the percentage of D₂O in the solvent from 90% to 10%. b) Simultaneous data fits to SDS 10% scattering data in varying H₂O:D₂O ratios, shown in Fig. 5, to an ellipsoid form factor and Hayter-Penfold MSA structure factor, detailed in Supplementary Information Section VI. (c) Structure factor \( S(q) \) and (d) form factor \( P(q) \) obtained from the ensemble fit and illustrative schematics of micelle shape and average structural analysis at varying contrast: SDS micelles

Selected 2D scattering patterns for the CV experiment ('full-scan') are shown in Fig. 6a, exhibiting a strong coherent signal at
large $\Delta p^2$ in D$_2$O-rich aqueous solution, a vanishing signal around $\Delta p^2 \sim 0$, and a weak signal approaching pure H$_2$O. Beyond the rapid, albeit coarse, analysis reported in the previous sections, detailed SANS data analysis can be carried out in order to obtain molecular and nanoscale information from the scattering objects, according to eq. (1). For this dataset, following data reduction and absolute calibration, each 2D pattern was radially-averaged and then fitted using SasView,$^{46}$ employing the well-known 'Ellipsoid Model' for the form factor $P(q)$ and the Hayter-Penfold mean spherical approximation ('HayterMSAStructure') for the structure factor $S(q)$ for screened electrostatic repulsions.

The same model was used to fit the entire set of curves, allowing for the 'scale' factor, the $\rho$ of the solvent mixture, and background $B$ to vary. Selected fits are shown in Fig. 6b and, for clarity of presentation, only one dataset is shown. Under the conditions studied, the micelles are well described by an ellipsoid with a 14 Å radius along the rotational axis, and 24 Å along the perpendicular direction. Details of the analysis and parameters obtained are given Supplementary Information section VI. The collective $S(q)$ and $P(q)$ obtained for the whole CV dataset is shown in Fig. 6c and d, respectively. The constrained fit of $N$ spectra, for a given $P(q)$ and $S(q)$, evidently increases the robustness and self-consistency of the analysis with respect to a single spectrum fit, and is thus also an asset of the proposed continuous microflow approach.

4 Conclusions

In this paper, we demonstrate a Small Angle Neutron Scattering (SANS) contrast variation (CV) and matching approach based on continuous flow microfluidics. Our approach addresses a major bottleneck in conventional SANS measurements of liquid mixtures and suspensions related to the sequential and discrete nature of the sample and measurement cell preparation and reutilisation. SANS is a powerful probe for the molecular and nanoscale study of simple and complex liquids, biological fluids and both organic and inorganic materials. As the flux of neutron facilities continually increases, and with the advent of next generation pulsed sources (e.g. European Spallation Source, ESS, due to go live within the next 2-6 years), pressure on more effective and precise sample environment will only increase. Microfluidics is evaluated due to its ability of precisely handling liquids, enabling mixing and composition variation of multicomponent mixtures at low Re numbers, desirable to minimise inadvertent flow-induced structural effects in systems of interest. Based on simple flow estimations of mixing time and Taylor-Aris dispersion, considering neutron beam size and flux, as well as sample contrast, we design a continuous flow approach which is successfully demonstrated with three model systems. The microflow-SANS is first validated with isotopic water mixtures to confirm residence time distributions and convolution of input flow composition and response in SANS. We then characterised a model colloid (or nanoparticle) of 12 nm diameter SiO$_2$ and the ubiquitous SDS surfactant, obtaining both the form and structure factors of the object and ensemble spatial arrangement. Representative measurements for triblock copolymer pluronic P127 are provided in Supplementary Information Section VII, Figs. S5-7. We showed the unique advantage of adaptive microflow systems that enable the zoom-in into a narrow region of interest, given the shallow composition dependence of SDS in water scattering, by a trivial variation of the phase space probed by the system. The data were validated against the traditional, discrete SANS procedure requiring the preparation of multiple distinct samples, and followed by a lengthy cleaning protocol.

By contrast with previously reported high-throughput microdevice platforms for bio-SAXS which sequentially formulate, measure at stopped-flow, and wash a given sample composition,$^{11,12}$ our approach relies on continuously scanning the composition space during SANS data acquisition. It therefore makes full use of the neutron beam, without deadtime during measurement (except during device wash and sample change cycles). Steady-state, continuous flow-focussing approaches to generate and scan concentration profiles in microflow-SAXS$^{8,14}$ require considerably smaller beamizes ($<<$mm) and relatively involved calculations or simulations of the composition map. Our method benefits also from an internal calibration of composition, made possible by decoupling the SANS 'background' that provides an independent and self-consistent validation of CV conditions. Further improvements are, of course, possible, for instance by incorporating an efficient mixer and thus shortening the SANS measurement position from injection, and minimising composition dispersion, but its simplicity and versatility are important assets.

Evidently, microflow SANS has limitations, at present, associated with finite neutron flux. These include the handling of viscous liquids, whose mixing times are long and currently incompatible with an online approach. Experimentally, we estimate the maximum reasonable viscosity of the fluids to be 100 cSt, that generates a pressure drop of 1.84 10$^6$ Pa in the 3 m long channel, at a total flowrate of 0.2 mL min$^{-1}$. Similarly, extremely low scattering systems are currently best handled discretely to prevent dispersion over long measurement times and large sample volumes. For instance, if the acquisition time required per data point is $>10$ min, then the volume required becomes $>20$ mL. However, the advent of neutron sources of exceptional brightness, including ESS, will undoubtedly expand the applicability of microflow SANS and permit the handling of low contrast, low volume samples, and/or with increased spatio-temporal resolution. Evidently, an effective cleaning protocol must be available (potentially at high flow rates and using aggressive solvents) within reasonable timescales. Further, the microdevice must have a suitably low scattering background and absorption (which can be readily met by the use of quartz, aluminium, silicon of $\leq$mm thickness or even thin $\sim 100$ µm conventional borosilicate glass).

In figure 7, we benchmark the microflow and discrete SANS approaches with respect to typical (a) required sample volume, (b) composition resolution and (c) overall preparation and measurement time. The required volume is reduced to 2.4 to 12.9 mL, considering a 30 min experiment at 0.05 mL min$^{-1}$ and a 60 min experiment at 0.2 mL min$^{-1}$ as extreme cases. The standard approach, requiring $\sim$3-10 mL, depending on sample contrast and accuracy required, is normally more reagent consuming. The resolution, calculated as the difference in percent composition between to consecutive measures, is typically 10 % in the traditional...
approach, whilst in continuous flow we can reach 0.83 % with a ramp of 120 steps. The biggest advantage is reduction of the required time: in the case of static cells, it is necessary to prepare 8 to 10 solutions (1 to 5 min each), load the cells (1 to 3 min), perform the experiment and clean the cuvettes (5 to 10 min each, plus 20 min in the oven). In the microflow SANS, the time is differently managed: sample preparation (1 to 5 min for each of the two solutions), loading (1 to 5 min each), experiment (stabilisation and ramp time) and cleaning (10 min). Overall, we believe that microflow-SANS offers impressive potential gains over the traditional, lengthy discrete sample approach, enabling fine composition scans and rapid screening of a large parameter space, commonly found in soft matter systems.

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