Pinhole mirror-based ultra-small angle light scattering setup for simultaneous measurement of scattering and transmission

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ABSTRACT

An ultra-small angle light scattering setup with the ability of simultaneous registration of scattered light by a charge-coupled device camera and the transmitted direct beam by a pin photodiode was developed. A pinhole mirror was used to reflect the scattered light; the transmitted direct beam was focused and passed through the central pinhole with a diameter of 500 μ m. Time-resolved static light scattering measurement was carried out over the angular range $0.2^{\circ} \le \theta \le 8.9^{\circ}$ with a time resolution of ~33 ms. The measured scattering pattern in the *q*-range between 5×10^{-5} and 1.5×10^{-3} nm⁻¹ enables investigating structures of few micrometers to submillimeter, where *q* is the scattering vector. A LabVIEW-based graphical user interface was developed, which integrates the data acquisition of the scattering pattern and the transmitted intensity. The Peltier temperature-controlled sample cells of varying thicknesses allow for a rapid temperature equilibration and minimization of multiple scattering. The spinodal decomposition for coacervation (phase separation) kinetics of an aqueous mixture of oppositely charged polyelectrolytes was demonstrated.

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I. INTRODUCTION

Small-angle light scattering (SALS) is commonly used to investigate the structure of complex fluids and biological systems in the length scales from a few hundred nanometers to micrometers. The corresponding scattering angle, θ , lies between a few degrees and ~10° within the forward scattering regime.^{1,2} For isotropic systems, the scattering data are presented as the change of the scattered intensity vs the momentum transfer, q (also known as the scattering vector), which is related to θ by

$$q = (4\pi n/\lambda)\sin(\theta/2), \tag{1}$$

with *n* being the refractive index of the medium and λ being the wavelength of the probe light. Thus, the detected *q*-range of SALS is between 10^{-4} and 10^{-3} nm⁻¹—about one decade lower than that of ultra-small-angle x-ray scattering (USAXS) or ultra-small-angle

x-ray neutron scattering (USANS), respectively. The length scales accessible by SALS enable the characterization of colloid sizes³ as well as their complex aggregation and gelation behavior.^{4–10} SALS was extensively used to measure the spherulitic morphology and kinetics of crystallization of polymers, ^{11–18} structure under flow and deformation, ^{19–26} kinetics of phase separation, ^{27–32} and microfluidics.^{33,34} Phase separation kinetics in colloid and polymer mixtures^{35–40} are well-suited for SALS, while, more recently, the effect of electrostatics on the liquid–liquid phase separation in proteins and charged polymers (polyelectrolytes)^{38,41,42} and mixtures of oppositely charged polyelectrolytes leads to coacervation with unique properties with phase behavior that mimics biomolecular condensation⁴⁰ and intracellular phase separation.⁴³ These areas provide rich opportunities for new applications of SALS methods.

A 2D photon detector is often used to register the scattering signal where the high angle scattering data are limited by the size

of the detector. Probing low angle scattering is challenging, owing to the inaccessible region setup by the beam stop. In addition, parasitic scattering becomes progressively dominant when approaching lower angles. Efforts have been taken to extend the low angle detection limit. For example, Ferri practiced minimizing the low-angle inaccessible region by using a focused beam and reflecting off the transmitted direct beam using a polished needle.¹ Such scattering geometry allows collecting low-angle scattering data down to 0.1°, able to probe the structures of tens of micrometers, such as macroscopic aggregates and phase-separated domains. The focused beam optics can effectively reduce the size of the direct beam, and a lowangle limit of 0.3° can be accomplished generally.⁴⁴ Using a specially manufactured needle is critical to further extend this limit. The extended low-angle SALS is referred to as ultra-small angle light scattering (USALS) by some authors, although the terms USALS and SALS are often used interchangeably, depending on the instrumentation details. Invaluable information about hierarchical structures at multiple length scales can be obtained when combining USALS with other complementary techniques, such as SAXS and USAXS as well as SANS and USANS.

The use of a charge-coupled device (CCD) detector has become a common practice in SALS. Such a detector allows examining anisotropic scattering patterns (often in the solid form), e.g., polymer films subjected to tensile stress.²³ Typically, scattering patterns can be collected by a CCD camera at a rate of tens of frames per second; higher acquisition speed can be accomplished by using a complementary metal-oxide-semiconductor (CMOS) detector.^{2,45,46} Scattering power of a sample in a SALS experiment is generally much higher as compared with that in an x-ray or neutron scattering experiment, allowing to collect high-quality scattering pattern at the full speed of the detector, which makes SALS an invaluable tool to study fast kinetic processes.

The scattered light can be registered using one of the two methods. In a simple detection scheme, the scattered light is first imaged on a semi-transparent screen (e.g., a piece of diffusing plate or Lambertian diffuser), and the image is captured by the detector.^{3,23,47} This method is suitable for detecting strongly scattered samples, particularly, for those able to produce distinct scattering features, such as well-resolved intensity maxima. However, the scattered intensity is attenuated, and the pattern smeared by the screen, which makes it incapable of investigating weakly scattered samples; the detector may also be placed off-axis and the image distortion corrected for the viewing angle.^{23,47} The other method is to acquire the scattered light with the detector directly.^{1,11,16,48} In this case, a set of collection lenses are needed to project the scattered light over a wide *q*-range to the small sensor area of the detector (typically a few millimeters in size).

Commercial availability of SALS/USALS setups is limited. Some polymer characterization instruments, such as gel permeation chromatography (GPC) and rheometer, are equipped with a light scattering accessory. The accessible *q*-range in these modules, however, is limited; particularly, the low-angle data below $\sim 5^{\circ}$ are generally not available due to the geometric restriction of the instrument. On the other hand, the advancement of semiconductor technology makes high-quality laser sources and detectors progressively inexpensive. With some effort, a laboratory-based SALS/USALS setup can be built, which can certainly add a new dimension to x-ray and neutron scattering data collected at large-scale facilities.

In this article, we describe the design and implementation of a USALS setup based on a pinhole mirror for the simultaneous measurement of sample scattering and turbidity. Scattering in an angular range between $\sim 0.2^{\circ}$ and $\sim 9^{\circ}$ can be well probed with a minimum time resolution of 33 ms. The application of the pinhole mirror bypasses the need for a beam stop or a reflecting needle to suppress the main beam or the use of a semitransparent imaging screen and the placement of a camera either in-line or inclined to measure the scattered image. A LabView-based graphic user interface (GUI) was developed to integrate the continuous acquisition of the 2D scattering pattern and the transmitted direct beam intensity, allowing a real-time data visualization and convenient adjustment of control parameters for data acquisition. A case study of the coacervation of an aqueous binary mixture of model anionic and cationic polyelectrolytes in the presence of added salt is presented in Sec. III. In this example, the scattered intensity increases by a factor of 10⁵ from the early stage of spinodal decomposition to the late stage of the phase separation. We demonstrate that, with a proper attenuation of laser intensity and reduction of detector exposure time during the progression of kinetics, it is feasible to track the complete phase separation process by employing a cost-effective CCD detector with a limited dynamic range (8-bit), though ideally a sophisticated (also much expensive) detector with a high dynamic range is more convenient.

II. EXPERIMENTAL SETUP

A. Optical layout

The optical layout of the USALS setup is shown in Fig. 1. A linearly polarized 17 mW He-Ne laser (REO Precision Optical Solutions; mode: TEM₀₀ > 99%, wavelength (λ): 632.8 nm, and polarization ratio: >500:1) with a beam diameter of 1.0 mm was used as the light source. All lenses are achromatic and anti-reflective. M1 and M2 are broadband dielectric mirrors for beam steering; together with the pinhole mirror M3, the entire setup was housed on a 120×75 cm² optical table. Two sets of neutral density filters (NDFs) are used to attenuate the incident beam (NDF1) and the transmitted beam (NDF2), respectively. The attenuation ratio can be adjusted during a scattering experiment, depending on the level of the scattered intensity. A spatial filter comprises lenses L1 and L2 with the focal lengths (f) of 30 and 50 mm, respectively; and a pinhole aperture (P1) with a diameter of 50 μ m was used to improve the beam homogeneity and to increase the beam diameter to 1.6 mm. An adjustable pinhole (P2) is used to remove pre-sample parasitic light.

The collimated beam from the spatial filter incidents on the sample cell, which is housed in a temperature-controlled sample holder. The details of the sample holders will be discussed later. The scattering volume is centered at the front focal point of lens L3 (f = 75 mm) and the pinhole on M3 (Lenox Laser, Inc.) at the back focal point. L3 has two functions: it parallelizes the scattered light and at 90° reflects it to the detection optics; in the meantime, the transmitted direct beam is focused at the pinhole of M3, being recorded by a silicon pin diode (DET100A2, Thorlabs) after passing through NDF2 and lens L6 (f = 40 mm).

The pinhole mirror M3 is made of aluminum with a flatness of 1–2 waves; the laser-drilled central hole has a diameter of 500 μ m.



FIG. 1. Optical layout of the USALS setup. Characteristics of the focused direct beam at the pinhole on M3 are schematically shown in the inset.

The beam waist radius of the direct beam at the pinhole of M3, w_0 , is 18.7 μ m, which is calculated using the following equation:

$$w_0 = f\lambda/\pi w,\tag{2}$$

with *f* being the focal length of L3 and *w* being the beam radius before L3 (0.8 mm after the spatial filter). The Rayleigh length z_R of the focused direct beam is 1.7 mm, which is calculated using the following equation:

$$z_R = \pi w_0^2 / \lambda. \tag{3}$$

A 500 μ m pinhole was used for the easiness of optical alignment. The current design provides an angular range of scattering between 0.19° and 8.6°, corresponding to a *q*-range between 5×10^{-5} and 1.5×10^{-3} nm⁻¹. The maximum scattering angle is truncated by the exterior size of L3 and M3, while the minimum angle by the size of the central pinhole on M3.

The scattered light is relayed to an 8-bit CCD detector (acA1300-30um, Basler AG) via lens L4 and L5 (f = 50 mm for both the lenses). L5 is directly mounted on the detector. The relative distance between the detector and L4 can be adjusted to achieve an optimal projection of the scattered light onto the imaging sensor ($4.9 \times 3.6 \text{ mm}^2$).

B. Sample environment

Proper choice of the sample cell is critical for accurate data collection. A flat glass cell is used to load a liquid sample; such a cell can be a commercially available rectangular cuvette or a home-constructed one for special applications as discussed below. Generally, maintaining a short optical path length of the sample is advantageous in a SALS measurement for two reasons. First, the entire sample volume along the pathway of the direct beam is subject to the scattering event, which causes uncertainties in the angle-dependence of the scattered intensity. This issue is avoided in a classic static light scattering (SLS) apparatus where a cylindric cell with a defining aperture being mounted right in front of the

detector effectively rejects scattered lights with a different momentum transfer from that being set by the goniometer. Such a screening mechanism is not implemented in our USALS instrument equipped with a 2D detector. One could, in principle, add a confocal component in the detection optics. However, it will substantially increase the difficulty of the optical alignment. Second, and more importantly, a thicker sample may significantly increase the chance of multiple scattering.

The sample cell with an optical path length being less than 500 μ m was made by sandwiching double-sided adhesive Scotch tapes with two pieces of 180 μ m-thick glass coverslip. The glass coverslips were precleaned using sulfuric acid with the addition of a small amount of glassware cleaner (NOCHROMIX, Godax Laboratories). The sample thickness is determined by the number of layers of the tape. The cell is sealed using the UV-curable optical adhesive (NOA 60, Norland Products, Inc.) to prevent loss of the solvent. Using such a thin cell is critical when measuring strongly scattered samples, as otherwise the multiple scattering effect will largely overshadow the scattering features. In addition, the thin cell allows a quick thermal equilibration, which is particularly useful when studying systems showing fast kinetics of phase separation.

A sample cell holder with a precise control of temperature -40-80 °C was designed to house the thin cell, as shown in Fig. 2. Heating and cooling are realized by a Peltier element (TE Technology) that is sandwiched between a circulation-based heat sink and a mounting plate. The heat sink, Peltier element, and mounting base are attached using ZnO thermal paste. A thermal sensor is installed on the mounting plate that is in direct contact with the thin cell. A piece of polyurethane foam tape (3M) of thickness 1.6 mm serves as a thermal insulator.

Alternatively, a temperature-controlled cuvette holder (Qpod 2e, Quantum Northwest, Inc.) can be used to house standard rectangular cuvette (1×1 cm²). The temperature range is between -30 and 110 °C controlled by a Peltier module. Weakly scattered samples, such as dilute colloidal suspensions, can be examined using such a sample manipulation system.



C. Data acquisition and processing

2D scattering patterns captured by the CCD detector is transferred to the computer via a USB3 cable. The output current of the pin diode is converted to voltage, which is acquired and transferred to the computer via a universal serial bus (USB) data acquisition board (NI DAQ6001, National Instruments). A LabVIEW GUI was developed for the control of CCD operation as well as a continuous visualization of the 2D scattering patterns and the intensity of the transmitted direct beam (see the supplementary material for details). The 2D scattering patterns are automatically converted to 1D profiles during acquisition, which provides a convenient in situ check of the intensity level and the scattering features. The user-defined time sequence of data acquisition can be programmed within the GUI, allowing to set up an optimized intensity level and time resolution for studying kinetic processes, meeting the capability of the CCD detector (see the supplementary material for details about the CCD characteristics).

If the 2D detector has only limited dynamic range, such as the one used in the current setup, it is critical to avoid pixel saturation of the detector, which can be easily identified from the appearance of hot spots on a 2D image and the corresponding 1D profile. Investigating a kinetic process, such as phase separation of polymer blends, calls for a special attention from the operator, as the intensity level becomes progressively stronger as the phase separation develops. It is possible to intervene such a time-resolved experiment to tune the intensity to a proper level by adjusting NDF1 (see Fig. 1) or by reducing the exposure time of the CCD during the course. Since the intensity of the transmitted direct beam is continuously recorded, 2D scattering patterns captured at different beam attenuations and CCD exposure times can be normalized and compared. Another technique to avoid pixel saturation, yet maintaining a good signalto-noise ratio of the scattering data, is to collect multiple images at a short exposure time and average them out.

Two backgrounds need to be properly subtracted from the scattering data of a sample. For isotropic samples, such subtraction is performed on the 1D profiles circularly averaged from the 2D images. An empty beam run is referred to as a measurement performed without the sample but with the laser on. A dark count of the CCD detector is measured when the laser is off. All backgrounds must be measured using an identical instrumental configuration with the same incident beam intensity and detector exposure time as used for measuring a sample. A general subtraction scheme is given in Eq. (4), where $I_{\rm cor}$ is the corrected 1D scattering profile; $I_{\rm sam}$, $I_{\rm em}$, and $I_{\rm dark}$ are the profiles of the sample, the empty beam, and the CCD dark count; and $C_{\rm sam}$ and $C_{\rm em}$ are the pin diode readouts of the sample and the empty beam. The corrected intensity profiles are normalized by the detector exposure time t and $C_{\rm em}$,

$$I_{\rm cor} = \frac{1}{tC_{\rm em}} \bigg[(I_{\rm sam} - I_{\rm dark}) - \frac{C_{\rm sam}}{C_{\rm em}} (I_{\rm em} - I_{\rm dark}) \bigg].$$
(4)

Note that scattering of the solvent often needs to be subtracted from that of a multi-component sample, such as a dilute colloidal suspension. A separate scattering measurement for the solvent needs to be carried out. In this case, the solvent is treated as a "sample" and the scattered profile needs to be corrected using Eq. (4) first, followed by the subtraction with a consideration of the volume ratio of the colloidal particles, ϕ , as given in the following equation:

$$I_{\rm cor} = I_{\rm cor}^{\rm (solution)} - (1 - \phi) I_{\rm cor}^{\rm (solvent)}.$$
 (5)

The conversion of a 2D scattering pattern to a 1D profile and averaging over multiple profiles are performed using a MATLAB program. The first step is to find the center of the patterns. A pinhole with a diameter of 200 μ m is placed at the sample position, and the concentric rings in the Airy disk [Fig. 3(a)] can be used for center calibration. Note that to produce the diffraction pattern shown in Fig. 3(a), pinhole mirror M3 is shifted to a slightly off-axis position (maintaining a 45° angle with respect to the direct beam) to reflect the central spot (attenuated) to the CCD detector. The circular averaged intensities are then plotted as a function of the pixel position that needs to be converted to scattering angles and *q*. The detector pixel position, *N*, is related θ by a linear coefficient *k*,

$$\theta = kN,$$
 (6)

which can be determined using various calibration standards. In the case of aforementioned Airy disk, the first intensity minimum appears at an angle

$$\theta_0 = 1.22\lambda/d,\tag{7}$$

which is 0.003 86 in radian (*d* is the diameter of the pinhole), corresponding to N = 89 on the detector; the coefficient so determined is $k = 4.337 \times 10^{-5}$. A diffraction grating of 250 lines/in. was used to cross check. Eight sharp diffraction peaks are resolved in the 2D detector [Fig. 3(b)]. The first order intensity maximum appears at N = 72 and is converted to an angle of 0.355° , which is consistent with the theoretical value. The scattering angles can be further converted to *q*, according to Eq. (1), with the consideration of the refractive index of the scattering medium.

A suspension of polystyrene spheres with an average diameter of 29.64 μ m (NIST SRM 1961) at a concentration of 0.025% by mass was measured. The particles were stabilized against sedimentation



FIG. 3. Diffraction patterns of a pinhole with a diameter of 200 μ m (a) and a grating of 250 lines/in. (b). The patterns are captured by the CCD detector and are used for scattering pattern calibration. See the text for details.

by using a mixture of H₂O and D₂O with a volume ratio of 1.04. Figure 4 shows the 1D scattering profile that is obtained by averaging 50 frames of the scattering patterns collected at an exposure time of 100 μ s. Scattering from the solvent was subtracted according to Eq. (5). A transmittance of 96% was determined by the ratio of the pin diode readout measured with and without the sample, suggesting that multiple scattering was largely prevented at this dilution. The 1D profile can be a model fit using Mie theory for the solid sphere with a consideration of diameter polydispersity following a Γ -distribution (see the supplementary material for fitting details). Note that for such large scattering entities as used in this demonstrative experiment, showing distinctive features in a scattering profile, it is important to smear the model with the beam profile to achieve a global fit of the curve. The fitting results in an average sphere diameter of 30.15 μ m with a standard deviation of 1.0 μ m.

III. EXAMPLE APPLICATION: PROBING THE PHASE SEPARATION OF OPPOSITELY CHARGED POLYELECTROLYTES

A case study of the spinodal decomposition in a solution of oppositely charged polyelectrolytes is discussed. The coacervation via associative phase separation was induced by increasing



FIG. 4. 1D scattering profile of a suspension of polystyrene spheres with an average diameter of 29.64 μ m, concentration of the suspension is 0.025% (mass fraction), dispersed in a mixture of H₂O and D₂O at a volume ratio of 1.04. A 10 mm-thick rectangular glass cuvette was used as the sample cell. The solid line is the fit using Mie theory for a solid sphere. See the text and supplementary material for details.

the temperature of an initial one-phase solution of the polyelectrolytes at an appropriate salt concentration.⁴⁹ Upon a temperaturejump into two-phase region, separation takes place leading to liquid-like microdroplets of dense coacervate polymer phase with an ultralow interfacial tension.^{50,51} A mixture of poly(potassiumstyrene sulfonate) (KPSS) with a weight average molecular weight M_w of 196 kg/mol and poly(diallyl dimethyl ammonium bromide) (PDADMAB) with M_w of 60 kg/mol was used as a model system. The homogeneous one-phase solution was prepared at room temperature. The total polymer concentration was $c_p = 0.3$ mol/l with a 1:1 charge stoichiometric ratio of monomers. KBr was added at a



FIG. 5. Transmittance as a function of the temperature of the aqueous mixture of KPSS and PDADMAB at a concentration of polymers $c_p=0.3$ mol/l and that of the added KBr $c_{KBr}=1.8$ mol/l. The cloud point $\mathcal{T}_{cp}=33.2\ ^\circ\text{C}$ is indicated by a vertical dotted line.

concentration of c_{KBr} = 1.8 mol/l. 30 µl of sample was loaded in a thin sample cell made of glass covers as described in Sec. II B with a sample thickness of 100 µm.

Cloud point measurement was performed by measuring transmittance of the sample using the pin diode during the increase of temperature at a rate of 0.2 °C/min. The thin sample cell is mounted on the home-built sample holder as described in Sec. II B. Figure 5 shows the transmittance change as a function of temperature; a dramatic decrease of transmittance is observed when the temperature is higher than the cloud point $T_{cp} = 33.2$ °C.

The sample was temperature jumped to 35.4 °C (into the unstable regime of the phase diagram⁵²) from room temperature, and the phase separation kinetics is monitored. Note that the small sample volume and the efficient thermal contact between the Peltier module



FIG. 6. (a) Transmission % through the sample during the progression of phase separation. The inset shows photodiode voltage as a function of time and incident intensities I_i applied during phase separation. The dotted line indicates the change in I_i from 232 to 52 μ W. (b) Normalized scattering profiles acquired at different times during phase separation.

and the thin cell allows the sample to equilibrate at the set temperature within ~10 s. Continuous data acquisition was triggered when the sample was mounted; in the data analysis stage, the time stamp of 15 s was set as the onset point. The development of the phase-separated domains caused a continuous increase of the turbidity of the sample, which is captured as a monotonical decrease of the transmittance with the progression of phase separation, as shown in Fig. 6. Note that NDF1 (see Fig. 1) was adjusted once during the experiment to avoid CCD pixel saturation in the later stage of phase separation due to the formation of large domains. Such adjustment is indicated in the inset of Fig. 6, where approximately fivefold drop of pin diode voltage readout appears at $t_w = 251$ s, caused by the attenuation of the direct beam. A continuous decay of transmittance, as it should be, is shown after intensity normalization.

In this experiment, the number of frames in a set for the profile averaging is changed from 5 frames (acquisition time is 165 ms) at the early stage to 20 frames (acquisition time is 660 ms) at the late stage when kinetics significantly slows down. We normalize the



FIG. 7. Time evolution of the normalized scattered profiles demonstrating phase separation during (a) early stages and (b) late stages of phase separation kinetics. Dotted lines indicate the locus of the scattering peak q_p as a function of time.

average scattering profiles with respect to transmission, exposure time, and the incident intensities, following Eq. (1). The data are presented in Figs. 6 and 7. The peak position, q_p , of the scattering profiles remains unchanged, and intensity exhibits q^{-2} power-law dependence at in the region $q > q_p$ for t < 71 s. The value of q_p slowly decreases, and the intensity exhibits q^{-4} -power law dependence for $q > q_p$ and t > 71 s. This is the well-known Porod law behavior, which characterizes the three-dimensional domain formation. The time evolution of the peak position clearly demonstrates a spinodal decomposition mechanism of phase separation.^{53–56} Additional details on the phase separation kinetics for the aqueous mixture of KPSS and PDADMAB will be presented in a separate publication.

The phase separation kinetics is monitored by capturing the time-dependent 2D scattering profile. For these measurements, we use an 8-bit CCD at an offset position so that the scattering center remains on one side of the detector, rather than in its center. The signal-to-noise ratio is improved by capturing multiple 2D frames within a short duration. The number of frames in a set for averaging is increased as the kinetics slows down with the progression of phase separation. We make sure that the change in the intensity during the acquisition of the set of frames for averaging is negligible. The scattered intensity increases with the progression of the phase separation. In a typical experiment, the laser light intensity and camera exposure time are, therefore, adjusted to avoid saturation of the camera sensor as it has a limited dynamic range.

IV. CONCLUSION

The USALS setup developed using a pinhole mirror simultaneously measures the scattering profile and transmission through samples. The design can accommodate different sample cells and holder based on the experimental requirements. The setup is suitable for measuring phase separation kinetics and structures between a few micrometers to a few hundreds of micrometers. These length scales are ideal to study the phase separation kinetics in an aqueous mixture of oppositely charged polyelectrolytes and more generally problems suitable to the formation of membraneless organelles and biomolecular condensation. A general experimental protocol is further presented to measure scattering from thick and thin samples by systematically adjusting camera exposure time and incident intensity during phase separation kinetics using a camera of a limited dynamic range.

SUPPLEMENTARY MATERIAL

See the supplementary material for details about (1) the LabVIEW-based GUI for data acquisition, (2) characteristics of the CCD camera, and (3) fitting the 1D scattering profile of the polystyrene sphere using Mie theory.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

DATA AVAILABLITY

The primary data that support the findings of this study are available within the article; The data that support the findings of this study are available from the corresponding author upon reasonable request.

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