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A critical examination of the components of the Schlieren effect in flow analysis

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Abstract

The Schlieren effect is a consequence of light inflexions altering the spectrophotometric measurements that are due to the formation of optical artefacts such as mirror and lenses inside the flowing sample. It may influence signal-to-noise ratio, sample throughput and measurement repeatability in spectrophotometric flow-based procedures. It was critically examined, in order to provide guidelines for system design. Results demonstrate that the magnitude of the Schlieren effect is governed by the combined influence of two components. The first is related to loss of light by reflection at sharp liquid interfaces and prevails under poor mixing conditions. In this situation the transmitted light becomes more intense and somewhat erratic. The second is related to light refraction in regions with pronounced refractive index gradients, and prevails under good mixing conditions. It can lead to positive or negative modifications of the transmitted light, the direction being dependent on the relevance of these components. The importance of the solubility of the chemical species involved, the possibility of interactions with the inner wall of the tubing, and the feasibility of dual-wavelength spectrophotometry for compensating purposes are discussed.

Keywords: Flow analysis; Schlieren; Refractive index; Spectrophotometry; Mixing conditions

1. Introduction

In the 17th century Robert Hooke investigated some optical properties related to the atmospheric environment and to aqueous solutions (see Appendix 1), and noted the deflection of a light beam passing through a medium with temperature gradients. Furthermore, he proposed the classical experiments demonstrating that both temperature and concentration gradients can alter light propagation [1]. This phenomenon is commonly observed in daily life and one practical example is the appearance of low-definition moving images above the surface of asphalt highways. The phenomenon has become known as the Schlieren effect [2]. The term "schliere" was introduced by Toepler in 1864 as "an inequality of the optical refraction of an otherwise homogeneous mass" [1]. In English language documents, terms such as "streak" and "stria" have also been used.

The Schlieren effect is also observed in liquid media, and can be a limiting factor in spectrophotometric measurements because the light deflection alters the intensity of the transmitted light beam. In this context, the influence of differences between the environment and sample temperatures on absorbance measurements has been reported for stopped-flow procedures [3]: The systematic deviation in absorbance was proportional to the temperature differences but also depended on instrument (optical) performance. The effect also manifests itself when mixing identical solutions at different temperatures, as noted by Miller and Gordon [4] who observed the formation and fading of a strong light-absorbing species during mixing, and reported a linear dependence of the measurement deviation with the temperature difference, as well as the formation of liquid lenses.

The Schlieren effect has also been reported in relation to HPLC [5] when a sample leaves a heated column and reaches

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the flow-through detector at a lower temperature. Concentration and temperature gradients inside the flowing sample can alter the absorbance measured because the resultant refracted index gradients lead to the formation of liquid lenses and the related optical disturbance can be compensated by dual-wavelength spectrophotometry [6].

It should be emphasised that the Schlieren effect is often associated with gradual variations in temperature and/or concentration, and hence refractive index. The effect is reproducible and somewhat wavelength dependent, as it is linked to the formation of relatively stable liquid lenses. It can therefore be exploited in a positive manner, e.g. when non-specific light scattering is used for the determination of a major analyte. In this context, the determination of glycol in aviation deicing/anti-icing fluids [7] and a flow-based strategy for verifying alcoholic beverage adulteration [8] are good examples. It has often been used as the measurement basis (Schlieren optics) for liquid chromatography [9–12] and enthalpimetric procedures involving optical beam deflection induced by the heat of reaction [13].

Limitations in spectrophotometric procedures due to the Schlieren effect are generally more pronounced in relation to flow analysis [14,15], because a perfectly mixed sample zone is not practically achievable, and an analyte concentration gradient is always present. Each fluid element of the sample is characterized by a different concentration, thus a different refraction index, and sharp changes in local concentrations and/or the presence of concentration gradients along the monitored sample zone give rise to the formation of relatively stable liquid lenses as well as a myriad of randomly distributed transient mirrors. These optical artefacts lead to undesirable fluctuations in the transmitted light that degrade the detection limit and measurement reproducibility.

In flow-injection analysis, the Schlieren effect was reported in 1977 in relation to the turbidimetric determination of sulphate in natural waters and plant digests: “a mixing boundary (Schlieren pattern) could be seen very clearly when the flow-through cuvette was removed from the spectrophotometer and observed visually after injecting a blank or a sample” [16]. The effect was also noted in 1978 when a LED-based device was implemented in a flow-injection system [17]. The authors removed the flow cell from its housing and verified with the naked eye that, during passage of the sample zone, the image seen through the flow cell was initially amplified and then reduced. This resulted in a reproducible signal profile with a positive peak followed by a negative peak.

From the above, it is possible to infer that the Schlieren effect in flow analysis is a consequence of the combined effects of two components. In this manuscript, the term “Schlieren” is always used regardless of whether the light beam is deflected by liquid lenses or mirrors. The aim of the work was to elucidate the effect of these two components, in order to provide guidelines for Schlieren compensation and for improving system design in flow analysis.

2. Experimental

2.1. Solutions

The solutions were prepared with distilled/deionised water and chemicals of analytical grade. The sample carrier stream (C in Fig. 1) and the solutions simulating the samples (0.0–10.0% (m/v) sucrose, 0.0–25.0% (v/v) ethanol, 0.0–14.0% (v/v) glycerol or 0.0–50.0 mg l^{-1} bromocresol green, BCG) were prepared in 0.01 mol l^{-1} $Na_2B_4O_7$ in order to avoid pH variations caused by sample dispersion, that could affect the BCG molar absorptivity [18]. Information on the refractive indices of the involved solutions is given elsewhere [14].

2.2. Instrumentation

The propelling device was a peristaltic pump (Ismatec IPC-04) furnished with Tygon pumping tubes. A loop-based injector was used for sample insertion into the flow system. Flow lines, reaction coils and sample loops were made from 0.8 mm i.d. polyethylene tubing of the non-collapsible wall type. The main reactor (R in Fig. 1) was made by winding the tubing around a 1.0 cm o.d. glass cylinder.

The spectrophotometer (Femto model 482) was furnished with a flow cell (Hellma 178.711-QS 30 μl inner volume, 10 mm optical path, tubular shape, parallel windows) and connected to a strip-chart recorder (Kipp & Zonen model 111). For comparative experiments a solid-state spectrophotometer (Ocean Optics USB 2000) furnished with optical fibres (i.d. 0.6 mm) attached to a transparent Z-shaped Plexiglas® flow-through cuvette (FIAlab 8 μl inner volume, 10 mm optical path, tubular geometry) was used. The latter spectrophotometer was operated through the FIAlab for Windows 5.0 software package with the integration time and measurement frequency set as 50 ms and 4.0 Hz, respectively.

Schlieren compensation by dual-wavelength spectrophotometry [14] was performed by real-time subtraction of the absorbance at the reference wavelength ($A_{\lambda2}$) from the absorbance at the analytical wavelength ($A_{\lambda1}$). Unless otherwise stated, analytical and reference wavelengths were 617 nm (λ_{max} for BCG) and 720 nm, respectively. Whenever required, spectra between 450 and 850 nm were recorded.

2.3. Procedures

The flow system was a linear configuration (Fig. 1) in which the sample was inserted directly into a carrier stream.

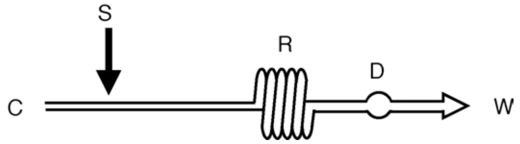

Fig. 1. Flow-injection manifold. S: sample; C: sample carrier stream; R: reactor; D: detector; W: waste collecting flask.

This generated a sample zone that underwent continuous dispersion while being transported towards the detector. As a consequence of the sample dispersion, concentration gradients were established along the flowing stream. The resultant transient variations of the transmitted light in the detector flow cell were used to explain the Schlieren effect and, when using BGC, to evaluate sample dispersion. All injections were made in triplicate.

The influence of sample volume (ca. 50–500 μl, 10–100 cm loop length), flow rate (1.5–5.0 ml min^{-1}) and reactor length (10–400 cm) were studied by separately injecting the dye solution (BCG), different solutions with a Schlieren-inducing species (sucrose, ethanol or glycerol) and mixed solutions containing both BCG and a Schlieren-inducing species.

The occurrence of a thermal Schlieren effect was also verified by inserting warm water (60 °C) into a water carrier stream equilibrated to room temperature (25 °C).

3. Results and discussion

BCG solutions were suitable for simulating samples because good linearity of BCG concentration versus recorded peak height function was always obtained for absorbances <2.0 ($r > 0.9999$; $n = 5$). Moreover, adsorption of the dye on the tubing inner walls was not observed and after 4 h working periods the main reactor (Fig. 1) remained colourless and there was no baseline drift.

3.1. The components of the Schlieren effect

Under good mixing conditions, refractive index variations along the sample zone are gradual, especially when the Schlieren-inducing species have good solubility, and the concentration gradients along the flowing sample are ideally true monotonic functions. As the flow analyser operates under a laminar flow regime [18], cylindrical concentric layers with different refractive indices are established along the sample zone, resulting in relatively stable liquid lenses, which refract light onto or away from the detector during sample passage through the flow-cell. Consequently, the monitored signal did not have abrupt variations (Fig. 2a) and the output signal was smooth and proportional to the concentration of the Schlieren-inducing species in the injected solution. Increasing sample dispersion reduced the intensity of the Schlieren effect but complete suppression was not obtained (see Fig. 2). It should be stressed that pronounced variations in recorded signal profiles were observed when using different flow-through cuvettes from different manufacturers and this result corroborates earlier reports that the Schlieren effect is highly dependent on the optical performance of the instrument [19].

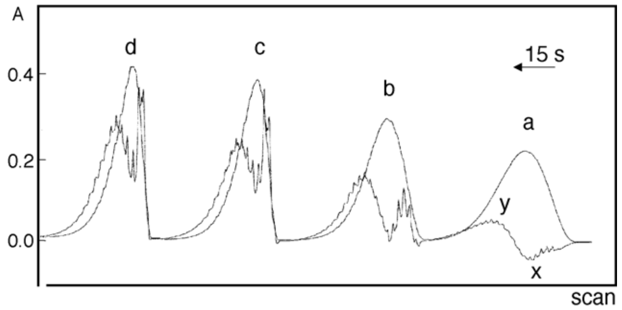

Fig. 2. Influence of reactor length using the flow system in Fig. 1 with the Femto spectrophotometer. S = 10.0% (m/v) sucrose (noisy trace) or 15.0 mg l^{-1} BCG (smooth trace) solution; injected volume = 100 μl, flow rate = 2.2 ml min^{-1}. Recorder tracings a, b, c, d correspond to reactor lengths of 240, 120, 60, 30 cm, respectively. Direction of trace from right to left.

The right-hand side of each peak of the recorder tracing in Fig. 2a refers to the front edge of the sample zone. The sample volumetric fraction [20] is higher for fluid elements near the centre of the tubing and lower for fluid elements near the tubing walls, and the reverse situation applies at the trailing edge of the flowing sample. The volumetric fraction of the carrier stream shows an analogous but opposite behaviour. On the assumption that the incident light beam of the spectrophotometer is not perfectly collimated, the concentric lenses will focus light onto or away from the detector, hence increasing or decreasing the measured (Fig. 2a). This aspect is herein referred to as component A of the Schlieren effect.

An important characteristic of the peak profile in Fig. 2a is the good repeatability, which is due to the good mixing conditions and the solubility of the Schlieren-inducing species. For 10 replicate injections relative standard deviations of ca. 4 and 2% were found for measurements performed at points x and y, respectively. The absorbance was then corrected by subtracting the apparent absorbance of the blank (Schlieren-inducing species) without significant error propagation effects. This approach has already been demonstrated in designing a flow-injection procedure for the determination of iodide in table salts [21].

In addition to the above observation, however, abrupt variations in the refractive indices of neighbouring fluid elements can result in a myriad of transient mirrors inside the flowing sample, which alter the power of the emergent beam of the spectrophotometer, leading to a noisy recorded signal (Fig. 2d). This effect, herein referred to as component B of the Schlieren effect, prevails under poor mixing conditions and/or when the Schlieren-inducing species has low solubility. Signal repeatability is therefore impaired and hence error propagation effects may limit the reliability of blank subtraction. The recorded signal has two bands, reflecting the regions of the flowing sample where concentration gradients are more pronounced. As mixing proceeds, the myriad of transient mirrors vanishes, reducing the intensity of this component of the Schlieren effect (Fig. 2). This has also been reported in relation to low absorbance spectrophotometry [22] where the Schlieren effect was minimised by adding an air bubble into the flow-through cuvette in order to improve mixing conditions, thus reducing the concentration gradients.

As light beam reflection is involved, the absorbance deviations tend to be positive. The transient mirrors may also direct stray light towards the detector, limiting the linearity of the signal/concentration curve in the region of higher absorbances. Furthermore, the effective optical path can be increased if the light beam reaches the detector after multiple reflections. This was confirmed by separately injecting 10.0% (m/v) sucrose + 30.0 mg l^{-1} BCG, 10.0% (m/v) sucrose and 30.0 mg l^{-1} BCG solutions (all in sodium tetraborate) into a flow system of Fig. 1 with the Femto spectrophotometer, a 100 μl sample volume, a 20 cm reactor length and a 5.0 ml min^{-1} flow rate. The peak area corresponding to (BCG + sucrose) mixed solution was 15% higher than the area summation of separate BCG and sucrose solutions. This effect also manifested itself when using ethanol or glycerol as the Schlieren-inducing species.

The recorded signals in Fig. 2 show periodic undulation due to the intermittent addition of small sample aliquots with different concentrations relative to the mean concentration inside the flow cell. If these additions affect the region traversed by the optical beam, they can result in regular absorbance fluctuations. These fluctuations are characterized by a fixed frequency, dictated by the peristaltic pump actuation, and can be removed by proper digital filtering [23].

With regard to the thermal Schlieren effect, only component A was observed, probably because heat transfer is a relatively slow process. This phenomenon is not relevant in flow analysis because temperature equilibrium is usually efficiently reached.

In summary, components A and B of the Schlieren effect are inherent to flow analysis, and their significance depends on whether stepwise or continuous changes in physical characteristics along the flowing sample are involved. Stepwise discontinuities relate to sharp interfacial differences between neighbouring fluid elements, whereas continuous changes relate to concentration gradients. It should be stressed that components A and B cannot be fully separated. Component A prevails under good mixing conditions and both can be observed, with dominance of component B, under inefficient mixing conditions. Furthermore, the “quality” of the incident light beam plays an important role in the Schlieren effect. The influence of component B was more evident with the Ocean Optics instrument in view of the better light beam collimation, whereas component A was enhanced for the Femto spectrophotometer. It should be stressed that this comparison involved the same flow-through cuvette.

Finally, it should be noted that the dominance of component A of the Schlieren effect was increased when an LED was used as the light source in the Ocean Optics spectrophotometer. This can be explained in terms of the larger solid angle associated to the incident light beam.

3.2. Influence of system dimensions

The sample volume introduced into the carrier stream plays a significant role in the Schlieren effect, especially with regard to component B. When a short reactor and a large sample volume (here 20 cm and 500 μl) were used, the front and trailing edges of the sample zone were more separated from each other, leading to the formation of two regions with pronounced concentration gradients and discontinuities. A longer residence time is associated with the sample trailing edge in relation to the front edge, lessening component B of the Schlieren effect and enhancing component A. In this situation, two noisy signals were recorded. The intensity of the Schlieren effect was negligible at the central portion of the sample. An example of this feature being exploited is a flow-injection system for the determination of chloride in battery electrolyte solution [24] in which only the central portion of the recorded signal was used for analytical purposes. Reducing the sample volume also reduces the distance between the two above-mentioned regions. For very low sample volumes, these regions coalesced causing the Schlieren noise to be located in the central portion of the sample zone.

Increasing the length of the main reactor can also lead to coalescence of these regions, as shown in Fig. 2. However, as the reactor length increases, component B of the Schlieren effect becomes less dominant. For very long reactors, therefore, component A of the Schlieren effect is dominant, and a smooth signal is observed (Fig. 2a). This effect can be explained in terms of the mean sample residence time in the analytical path. As this parameter was increased the available time for diffusion, and hence extinction of the transient mirrors [14], was increased (Fig. 3).

Another possibility for attaining different mean sample residence times inside the analytical path is to modify the flow rate. Decreasing this parameter led to a decrease in the intensity of the Schlieren effect under all investigated conditions (Fig. 4), regardless of the prevailing component (A or B). It is important to note that the Schlieren effect vanishes if the sample zone is stopped inside the flow-through detector for a long time interval [14] but this has not yet been exploited for real sample analyses.

3.3. Compensating the Schlieren effect

Dual-wavelength spectrophotometry [23] is an excellent option for compensating the Schlieren effect in flow analysis

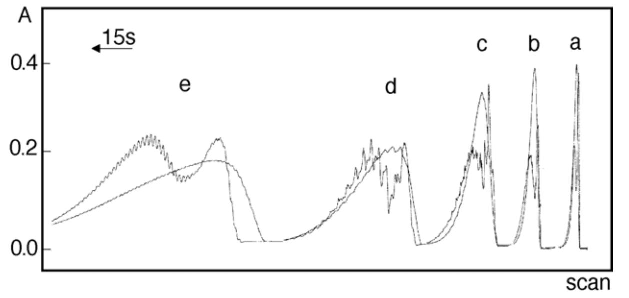

Fig. 3. Influence of flow rate. Reactor length = 30 cm; other conditions as in Fig. 2. Recorder tracings a, b, c, d and e correspond to 8.8, 4.8, 2.2, 1.2 and 0.6 ml min^{-1} flow rates, respectively. Direction of trace from right to left.

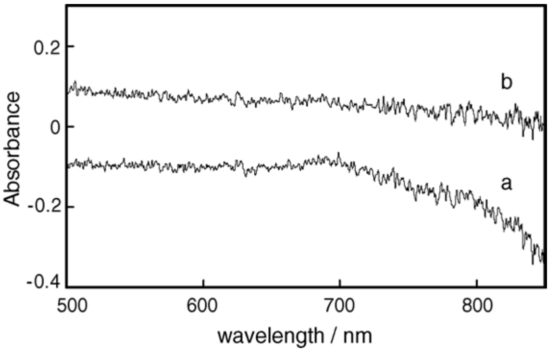

Fig. 4. Recorded "spectra" obtained using the Ocean Optics spectrophotometer. Spectra a and b relate to sample regions corresponding to minimum and maximum absorbance values respectively (50 and 170 s after sample injection); S = 50 µl of 10.0% (m/v) sucrose solution, reactor length = 400 cm; flow rate = 4.5 ml min^{-1}.

[14,25,26]. It is complementary to the other strategies already proposed for compensation such as sample/carrier matrix matching [19,27], reversed flow [28], nested-loop injection [29], monosegmented flow [30], reflective flow cells [31,32] and larger sample volumes [33]. Its efficiency for circumventing the Schlieren effect depends on whether component A or B is dominant.

For component A, tubular concentric lenses are established, causing light refraction, and hence Snell's law is applicable. In this regard, it was verified that light attenuation was more pronounced at higher wavelengths (Fig. 4a). Therefore, a scaling factor to normalize the Schlieren effect related to the different wavelengths could be used. On the other hand, light reflection prevailed in situations where component B was dominant because a wavelength-independent light attenuation was observed, leading to a "white" spectrum (Fig. 4b). In this situation, quantitative Schlieren compensation was achieved which was somewhat unexpected because white spectrum is not strictly attained. This is because the transient mirrors established between fluid elements of different refractive indices are not ideal and the incident light is also partially refracted, with the refraction angle being wavelength dependent. Here, it should be added that recorder tracings in Fig. 4 are somewhat noisy in view of the very low integration time (50 ms).

Regarding Schlieren compensation by dual-wavelength spectrophotometry, better efficiency was observed in relation to component B (Fig. 5), as the related spectrum approached an ideal white spectrum. Paradoxically, it has often been emphasised that the Schlieren effect becomes less pronounced as mixing conditions are improved (see also Fig. 2). However, under very good mixing conditions, component A of the Schlieren effect is dominant, and hence the efficiency

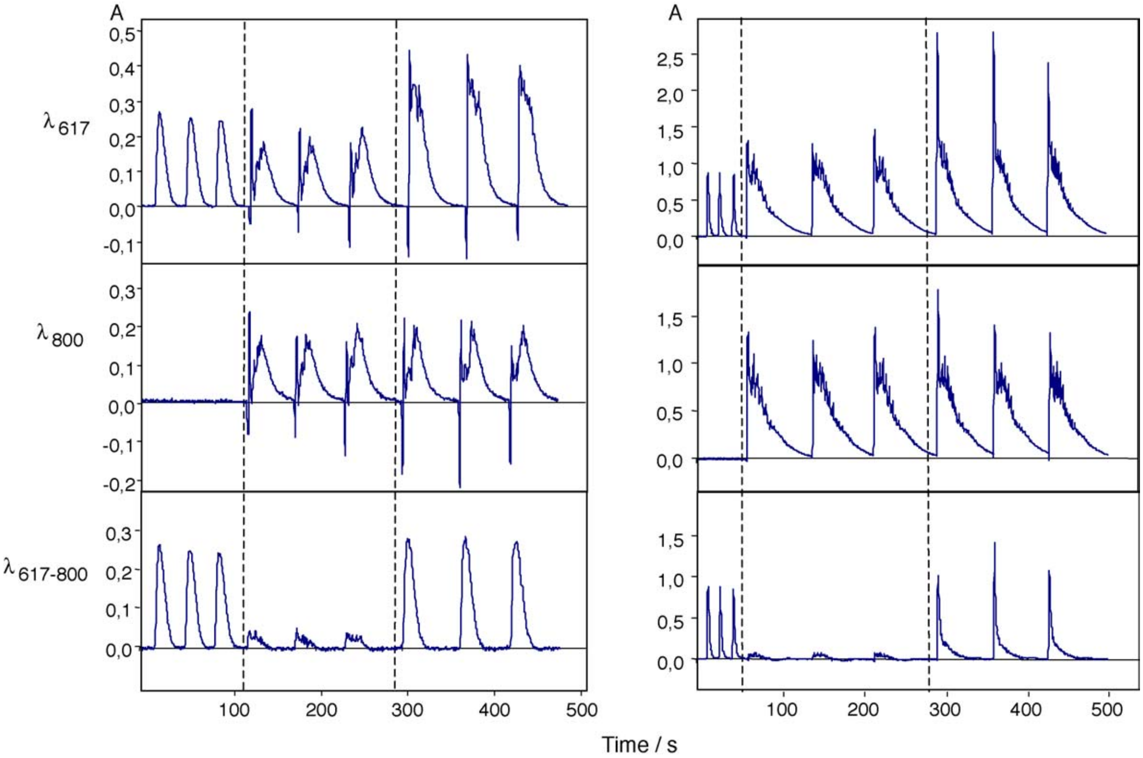

Fig. 5. Schlieren compensation under different conditions using the Ocean Optics spectrophotometer. Flow rate = 5.0 ml min^{-1}; sample volume = 100 µl; reactor length = 400 cm (left) or 20 cm (right). Each set corresponds to a triplicate processing of the sample (30 mg l^{-1} BCG), the Schlieren-inducing species (10% (m/v) sucrose), and the combined (30 mg l^{-1} BCG plus 10% (m/v) sucrose) solutions. All solutions were made up in 0.01 mol l^{-1} $Na_2B_4O_7$. Upper trace: analytical wavelength; middle trace: reference wavelength; lower trace corrected values by dual-wavelength spectrophotometry.

Table 1
Features related to both components of the Schlieren effect

	Dominant component	
	A	B
Mixing conditions[a]	Good	Poor
Optical element	Liquid lenses	Myriad of mirrors
Stability	Relatively steady	Transient
Geometry	Well-defined	Fuzzy
Repeatability	High	Low
Characteristic	Blank	Noise
Compensation by dual-wavelength spectrophotometry	Partial	Quantitative

[a] Including solubility of the Schlieren-inducing species.

of dual-wavelength spectrophotometry is somewhat reduced in view of the spectral characteristics involved (see also Fig. 4). For the successful application of dual-wavelength spectrophotometry, a compromise involving mixing conditions and spectral characteristics should be considered. For system design therefore, analysis of the Schlieren effect caused by particular species is recommended before a final decision on the extent of sample mixing can be made.

4. Conclusions

The Schlieren effect is often the limiting factor in maximising the signal-to-noise ratio in flow analysis. Although frequently discussed, the effect is not fully understood or characterized. It is determined by the combined influence of two components, and also depends on the characteristics of the incident light beam (especially with regard to collimation) as well as the optical performance of the spectrophotometer. Depending on the dominance of component A or B, transient liquid lenses or mirrors are established in a well-defined or random fashion, yielding either an organized or fuzzy geometry, resulting in high or low measurement repeatability, respectively (Table 1).

Additional experiments revealed that the effect is strongly dependent on the fluidic conditions (e.g. constant or pulsed flow, open tubular, single bead string reactor or knitted reactor) and on the solubility of the Schlieren-inducing species.

Acknowledgements

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Appendix A

Excerpts from the Hooke's *Micrographia* [34]. Comments in italic.

"A new property in the air and several others transparent mediums nam'd Inflection, whereby very many considerable Phenomena are attempted to be solv'd, and divers other uses and hinted." *About this observation he concluded*: "and upon the whole, I find much reason to think, that the true cause of all these Phenomena is from the inflection, or multiplicate refraction of those Rays of light within the body of the atmosphere, and that it does not proceed from a refraction caus'd by any terminating superficies of the air above, nor from any such exactly defin'd superficies within the body of the atmosphere." *And, later on*: "This inflection (if I may so call it) I imagine to be nothing else, but a multiplicate refraction, caused by the unequal density of the constituent parts of the medium, whereby the motion, action or progress of the Ray of the light is hindered from proceeding in a straight line, and inflected by a curve."

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