Technical Note:
TN-MR-FCGO1011

Turbidometric performance of Thermo Scientific Multiskan FC and Multiskan GO

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Abstract

Turbidometry is a special type of photometric measurement, where scattering of light caused by solid particles in the solution is measured. Turbidometry can be applied in a variety of research areas, for example, water and beverage quality control and microbial growth. It is based on the principle that all particles, including microbes, cause light scattering. The amount of light scattered is directly proportional to the amount of particles present in the liquid, when the shape and size of the particles remains the same.

Turbidity is commonly measured using special turbidometer/nephelometer devices, but it can also be determined using a normal microplate photometer or spectrophotometer.

Formazine solutions are traditionally used to verify and calibrate turbidometric devices on account of their high reproducibility. This note describes the verification of the turbidometric performance of Thermo Scientific Multiskan FC microplate spectrophotometer and Multiskan GO photometer with a standard formazine solution.

Introduction

Light absorbance is a completely different phenomenon than light scattering. Photometers may not be optimal for turbidometric assays, but most of them are nevertheless suitable for that purpose. Nephelometers are more sensitive in turbidometric measurements, but cannot be used for measuring absorbance. The basic operating principles of a microplate photometer in absorbance mode and turbidometric mode, a microplate nephelometer and a traditional cuvette nephelometer are shown in Figure 1.

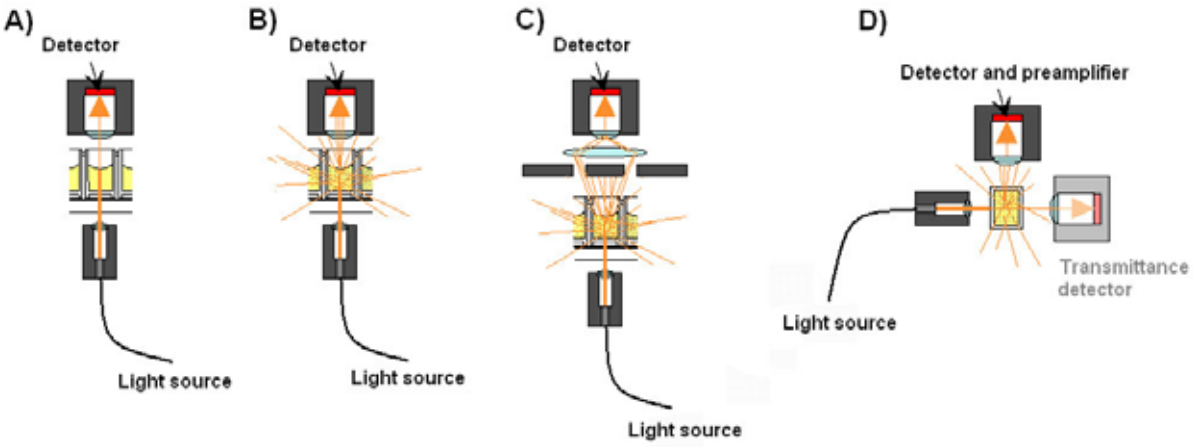


Figure 1. Operating principles of three different devices in turbidometric assays: A) a microplate photometer in absorption measurement, B) a microplate photometer in turbidometric measurement, C) a microplate nephelometer, and a D) traditional cuvette nephelometer.

Materials & Methods

1) Instruments

- Thermo Scientific Multiskan GO microplate spectrophotometer, product code 51119300
- Thermo Scientific Multiskan FC microplate photometer, product code 51119100
- A reference spectrophotometer

2) Consumables

Turbidometric performance was tested with the following microplates:

- Thermo Scientific Microtiter 384-well plate, clear, product code 8555
- Thermo Scientific NUNC optical bottom 96-well plate, polymer bottom, black walls, product code 165305
- Thermo Scientific NUNC optical bottom 96-well plate, glass bottom, black walls, product code 164588

The following cuvette was also used with the Multiskan® GO and the reference instrument:

- Brand Semi-Micro Cuvette, polyacrylic plastic, product code 759150

Turbidometric performance for both linearity and detection limit, was determined according to the principles described in the US EPA 180.1 standard. The testing was conducted with a defined standard solution (Formazine Standard Solution 4000 NTU, CamLab UK Ltd). The standard solution was diluted in three steps to obtain the turbidometric calibration series within a range of 0.2–4000 NTU.

The diluted samples were pipetted into different microplates in eight replicates and into semi-micro cuvettes in two replicates and then measured with Multiskan GO (microplate and cuvette) and Multiskan FC (microplates). The volumes used were 300 µl on 96-well plates and 50 µl on the 384-well plate. All plates and cuvettes were measured at 340 nm, 450 nm and 620 nm, respectively.
Linearity and detection limits were calculated from the data. A commonly used cuvette spectrophotometer was used as a reference instrument for the cuvette measurements.

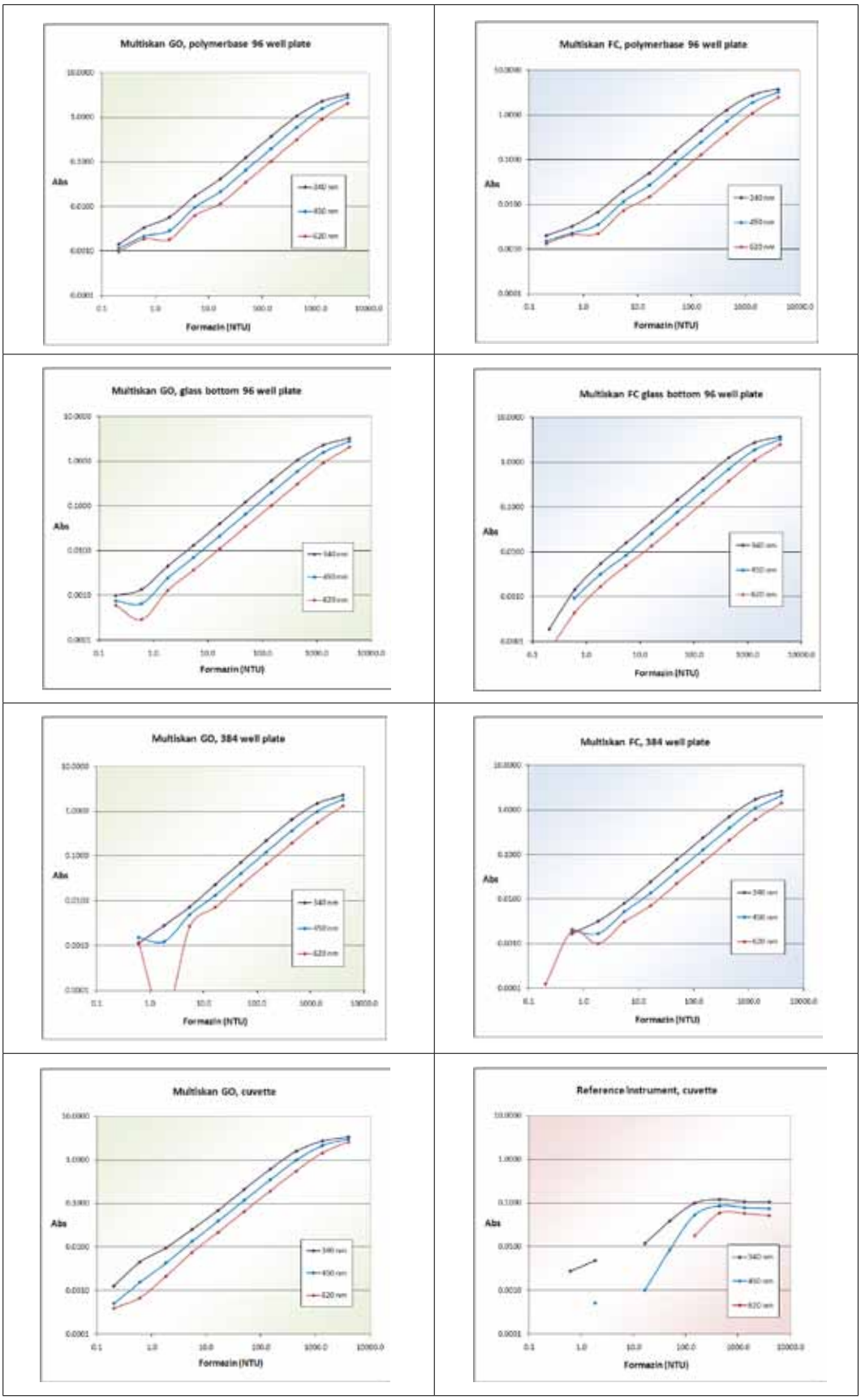
Results & Discussion

The calculated limit of detection (LOD) and linearity results using the Multiskan FC, Multiskan GO and the reference cuvette spectrophotometer are presented in Table 1.

Table 1. Limit of detection and linearity results for the Multiskan FC, the Multiskan GO and the reference instrument. The range is considered linear if the measured absorbance differs less than 10% from the calculated absorbance. The linear range is reported in number of serial dilutions. The actual values of the dilutions are also reported in parenthesis.

Microplate / Cuvette	Volume (ul)	Wave-length (nm)	Multiskan FC		Multiskan GO		Reference spectrophotometer	
			LOD (NTU)	Linear range in number of dilutions (NTU)	LOD (NTU)	Linear range in number of dilutions (NTU)	LOD (NTU)	Linear range in number of dilutions (NTU)
NUNC clear bottom black 96, polymer bottom	300	340	0.4	4 (16-444)	0.6	4 (16-444)	-	-
		450	0.9	4 (16-444)	0.8	4 (16-444)	-	-
		620	1.1	4 (16-444)	1.2	5 (16-1333)	-	-
NUNC clear bottom black 96, glass bottom	300	340	0.6	6 (1.8-444)	0.2	7 (0.6-444)	-	-
		450	0.4	8 (0.6-1333)	0.4	6 (1.8-444)	-	-
		620	1.7	7 (1.8-1333)	0.8	7 (1.8-1333)	-	-
Microtiter 384, clear	50	340	3.4	5 (5.5-444)	2.8	6 (1.8-444)	-	-
		450	6.0	6 (5.5-1333)	4.3	6 (5.5-1333)	-	-
		620	10.8	5 (16.5-1333)	5.6	6 (5.5-1333)	-	-
Brand Semi-Micro Cuvette, polyacrylic plastic	500	340	-	-	0.3	4 (5.5-148)	16.7	2 (16.5-49.3)
		450	-	-	0.1	8 (0.2-444)	41.2	1
		620	-	-	0.4	5 (5.5-444)	185.0	1

Figure 2. Examples of dilution curves of formazine series on 96-well glass and plastic bottom plates, a 384-well plastic plate and cuvette. The results of the three different instruments are presented on different backgrounds. Blue indicates the Multiskan FC, green the Multiskan GO and red the reference spectrophotometer.



The turbidity measurements are always performed with heterogeneous samples. Therefore, assay precision, normally expected from photometric measurements cannot be expected from turbidometric assays. Similarly, the linear measurement range is always decreased due to multiple scattering at high particle densities. In view of these well-known limitations the performance of the tested Multiskans with a linear measurement range of 1.5–2 OD units in turbidometric assays can be considered exceptionally good.
Based on these results, the glass-bottom plate performed slightly better in these tests than the plastic-bottom plate. It shows both a lower detection limit and a wider linear range with both instruments.
Cuvette measurements showed somewhat better performance than any of the microplate assays. There is also a difference between the plate matrixes with the 96-well plates showed better performance compared to the 384-well plate. The reason for this is probably the longer optical pathlength and therefore stronger light scattering.
In this test the reference cuvette spectrophotometer proved to be totally unsuitable for turbidometric measurements.

Conclusion

Both tested Multiskan instruments in this study showed very good performance in turbidometry with the formazin measurement.

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