Supelco®
Analytical Products

Application Note

McFarland Scale - Determination of Microbial Cell Concentration in Suspensions

Direct spectrophotometric measurement at 600 nm

Introduction

In microbiology labs it is important to know the concentration of microbial cells in suspensions e.g. in inoculum culture. The traditional way to determine the concentration by plating requires a significant amount of time. An alternative, rapid, low-cost and non-destructive way for the determination is the use of the McFarland scale. The McFarland scale corresponds to specific concentrations of CFU/ml of gram negative bacteria (e.g. *E.coli*). It can be used to estimate the cell concentration visually by use of specific McFarland standards (barium sulfate suspensions) or spectrophotometrically [1].

In the spectrophotometric measurement, the attenuation of the light intensity caused by the cells present in the suspension is determined by measuring the absorption and compared with the absorption measurement of McFarland standards under same conditions. The attenuation of the light intensity is primarily due to the scatter of the light and only partly to the absorption of the light by the cells itself.

Experimental

This Application Note describes the determination of the cell concentration in suspensions based on the McFarland scale.

The method can be used to yield a simple and swift estimate of the cell number. This method is preprogrammed in the Spectroquant® Prove 300/300 plus and Prove 600/600plus UV/VIS spectrophotometers and in the Spectroquant® Prove 100/100 plus VIS spectrophotometer with firmware version 1.5 or above. No further reagents are required to run this method.

Method

Spectrophotometric measurement at 600 nm

Measuring range

0.0 – 10.0 McFarland
0 – 3000 CFU (x 10^6/ml) based on *E.coli* [1]

Sample material

Bacterial suspensions, inoculum

MERCK

Reagents and auxiliaries

Cat. No.	Description
Instruments	
1.73028	Spectroquant® UV/VIS Spectrophotometer Prove 600 plus
1.73027	Spectroquant® UV/VIS Spectrophotometer Prove 300 plus
1.73026	Spectroquant® VIS Spectrophotometer Prove 100 plus
Software for data maintenance	
The Spectroquant® Prove Connect to LIMS software package provides an easy way to transfer your data into a preexisting LIMS system. This software can be purchased under:	
Y11086	Prove Connect to LIMS
Materials	
114946	Rectangular cell 10 mm (glass) or
Z801216	Semi-micro rectangular cell 10 mm (glass)* or
C5416	Semi-micro rectangular cell 10 mm (polystyrene)*
100921	Diethyl ether for analysis EMSURE®
PHR1239	ß-Carotene Pharmaceutical Secondary Standard; CRM

Also first generation Prove instruments are compatible and preprogrammed with this method.

* Due to the optical characteristics of the Prove Spectrophotometers the use of 10 mm micro cells is not possible. Plus, due to the automatic cell detection of the Prove instruments it is important to use semi-micro cells with complete side walls.

Analytical approach

Sample Preparation

- Homogenize samples by swirling carefully.
- Avoid shaking the sample too vigorously, since this may lead to damages of the cells.

Measurement

- Open the method list of the photometer and select method No. 2513 “McFarland”.
- The photometer automatically prompts a zero adjustment.

  It is recommended to use the same cell for zero adjustment and for sample measurement or a cell with identical optical properties and an identical absorption (matched pair).
- For the zero adjustment fill a 10-mm rectangular cell with distilled water and insert the cell into the cell compartment. The zero adjustment is executed automatically. Confirm the zero adjustment by tapping <OK>. The zero adjustment is valid for the entire measurement series
- Fill the sample solution into a 10-mm rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Confirm the measurement by clicking on <OK>. The measurement result appears in the display.

Data transfer from Prove spectrophotometers (optional)

After measurement transfer the values measured on the Prove spectrophotometer using the software “Prove Connect to LIMS”.

Note

The McFarland scale corresponds to specific concentrations of CFU/ml of gram negative bacteria (e.g. *E.coli*). If other organisms are used, the estimation becomes uncertain and the results should be verified by plating and determining the viable count [1].

Conclusion

This method offers a swift and simple alternative for estimating the cell concentration in suspensions. The measurement can be performed without high instrumental expense or additional reagents.

The method is preprogrammed in the Spectroquant® Prove 100/100 plus, Prove 300/300 plus, and Prove 600/600 plus spectrophotometers.

For more information
SigmaAldrich.com/photometry

References

1 Sutton, Scott. Measurement of Cell Concentration in Suspension by Optical Density. Journal of GXP Compliance 15:3 (2011), 49-53

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