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Original Article

Comparison of polystyrene versus cycloolefin microplates in absorbance measurements in the UV/VIS region of the spectrum

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Abstract

Background and aims: Polystyrene microplates are generally used to specify the absorption in the visible light region of the spectrum. However, they are capable of absorption in the ultraviolet (UV) range of the spectrum while they are not suitable for UV spectroscopy analysis. This study aimed to compare polystyrene and cycloolefin microplates for their background absorbance characteristics in the UV/VIS region of the spectrum.

Methods: Background absorbance of the mentioned microplates was measured using two different spectrophotometers and four various samples.

Results: The analysis of our results verified the advantage of applying cycloolefin microplate over polystyrene one for absorbance measurements in the UV range of the spectrum.

Conclusion: In general, suitable microplate selection is a critical factor in absorbance measurements, especially in the UV portion of the spectrum.

Keywords: Absorbance measurements; Polystyrene; Cycloolefin microplate; Spectrum

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Introduction

UV/VIS is a subclass of spectroscopy which uses visible light and adjacent near ultraviolet (UV) ranges for determining the concentrations and the characterization of dissolved substances (1-11). This spectroscopy is usually conducted in quart glass cuvettes. However, cuvettes do not provide sufficient throughput when dealing with large amounts of samples, small volumes of solutions, and toxic samples. In these cases, microplates can be used to speed up the work (12,13). Polystyrene microplates are generally utilized to determine the absorption in the visible light portion of the spectrum. Although they can be absorbed in the UV range of the spectrum, they are not suitable for UV spectroscopy analysis (14). Since polystyrene microplates are not capable of efficient light absorbance in the visible light region of the electromagnetic spectrum, the background absorbance by the microplate itself during microplate-based assays has not been of importance. In cases where the background absorbance does exist, dual wavelength measurements are applied to correct the background. In this correction method, it is assumed that the microplate has consistent background absorption at the two different used wavelengths. Nevertheless, this is not true with the most microplates in the UV range of the spectrum and choosing the plates is important for performing the measurements (15-17). Therefore, the aim of this study was to evaluate the background absorbance characteristics of polystyrene microplates which are commonly used in most of the microplate-based assays without taking enough care regarding choosing the appropriate microplate. For this purpose, the background absorbance features of polystyrene microplate in the UV/VIS region of the spectrum were evaluated and compared to a microplate made of a different polymer.

Materials and Methods

Effect of different devices on absorption rate

Two different 96-well microplates with various constituent

substances were examined including polystyrene and cyclic olefin copolymer (cycloolefin). In addition, the absorbance measurements were conducted to evaluate the effects of different devices on the absorption rate of each empty microplate. To this aim, two different instruments including Epoch microplate spectrophotometer (BioTek Instruments, Winooski, VT) and Synergy 4 hybrid multi-mode reader (BioTek Instruments, Winooski, VT) were used, along with Gen5™ software, version 2.00.18 (BioTek Instruments, Winooski, VT) running on external PCs to control the readers function and data capture. Two end-point absorbance measurements at 250 and 280 nm were performed before the spectral analysis of the microplates. Measurable wavelength spectrum for the first device was from 200 to 1000 nm, and for the second reader was from 300 to 800 nm for polystyrene plate and 280 to 700 nm for cycloolefin microplate, all in 10 nm increments.

Effect of different samples on absorption rate

Then, another experiment was designed to investigate the effects of different samples on the absorption level of the mentioned microplates. Four different samples were selected and analyzed using Epoch microplate spectrophotometer. To this end, individual wells of each microplate were filled with 200 µL of distilled water, fetal bovine serum, 0.5 mg/mL tissue plasminogen activator, and 2.5 mM L-cysteine in duplicate and then the absorbance was determined from 200 nm to 1000 nm in 10 nm increments.

Results

Effect of different devices on absorption rate

The background absorbance measurements of polystyrene and cycloolefin microplates were performed using two different instruments with various sensitivity of the measurement. The results are shown in Figure 1.

Following the application of Epoch microplate spectrophotometer (Figure 1A), the polystyrene plate exhibited a background absorbance at 230 nm of 3.876, showing the maximal absorbance value for this reader. This plate is practically canescent to the light of wavelengths 200 nm to 290 nm and its background absorbance decreases rapidly with wavelengths above 290 nm to a value of 0.752 at 300 nm and less than 0.070 by 390 nm. When wavelengths from 300 nm to 990 nm were tested, the polystyrene microplate background reduced slowly from 0.752 to 0.044. The cycloolefin microplate has a background absorbance of nearly 0.988 at 200 nm and slowly reduces to 0.097 by 230 nm and is 0.067 by 260 nm. In this study, the background absorbance in the visible light wavelengths was nearly 0.038.

Because of the high background absorbance of polystyrene microplate, Synergy 4 hybrid multi-mode reader could not provide the background absorbance value at 250 nm and showed the overflow message. The observed background absorbance was 3.323 at 280 nm for polystyrene microplate, which represented the maximal absorbance value for this reader. Further, background absorbance values for cycloolefin microplate were 0.076 and 0.053 at 250 and 280 nm, respectively. Considering the limitation of the Synergy 4 hybrid multi-mode reader for measuring the absorbance values, the detectable measurement range of the spectrum was more limited in comparison to the first device. Using Synergy 4 hybrid multi-mode reader (Figure 1B), the results of our measurement showed that the polystyrene plate exhibit a background absorbance at 300 nm of 0.785. It is practically canescent to the light of wavelengths 200 nm to 290 nm, with the background absorbance decreasing rapidly with wavelengths above 290 nm to a value of 0.785 at 300 nm and less than 0.066 by 390 nm. When the wavelengths from 300 nm to 700 nm were tested, the polystyrene microplate background reduced slowly from 0.785 to 0.042. The cycloolefin microplate has a background absorbance of nearly 0.053 at 280 nm and slowly reduces to 0.039 by 390 nm. Based on the results, the background absorbance in the visible light wavelengths was nearly 0.036.

Effect of different samples on absorption rate

The absorption pattern of four samples including distilled water, L-cysteine, and two protein samples were tested employing Epoch microplate spectrophotometer. The obtained absorbance values of polystyrene microplate wells containing distilled water (Figure 2A) were the same as the empty ones, while for the cycloolefin microplate, these values were a little more than the empty wells,

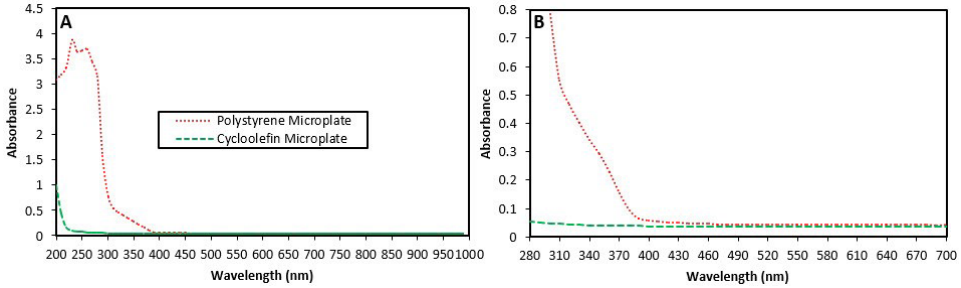

Figure 1. Spectral analysis of empty polystyrene and cycloolefin microplates using two different instruments: (A) Epoch microplate spectrophotometer and (B) Synergy 4 hybrid multi-mode reader.

especially in 200 to 310 nm. Furthermore, L-cysteine (Figure 2B) revealed its maximum absorbance at 210 nm of 2.734 in cycloolefin microplate. Then, it quickly decreased to 0.959 by 230 nm and was 0.092 by 280 nm, while the corresponding values in polystyrene microplate were 3.209, 3.925, and 3.227, respectively. Fetal bovine serum, as a high protein content sample, represented more complex patterns (Figure 2C). Moreover, both polystyrene and cycloolefin microplates had similar values in the range of 200 to 230 nm. Maximum absorbance values were observed at 230 nm for both microplates, but the difference is that the value related to polystyrene microplate was overflow and we had to insert a high value in order to draw the curve. Additionally, the absorbance values of the sample at 280 nm were found to be 3.465 and 3.119 for polystyrene and cycloolefin microplates, respectively. In addition, fetal bovine serum (FBS) had a relatively high absorbance up to 290 nm, and then it quickly decreases by 320 nm and 310 nm for polystyrene and cycloolefin microplates. A distinct peak was detected at 410 nm in both microplates, which is related to one of the FBS components. The corresponding values were the same for both microplates because of the negligible background absorbance of polystyrene microplate in the visible portion of the spectrum. Finally, a solution of 0.5 mg/mL tissue plasminogen activator was used as a single protein containing the sample (Figure 2D). The obtained spectrum of the cycloolefin microplate consisted of two distinct peaks, which were related to the absorbance values at 230 and 280 nm. These values were 2.172 and 0.456 for cycloolefin microplate, as well as 3.897 and 3.221 for polystyrene microplate.

Discussion

Several different factors should be considered before selecting the microplate. Although quartz cuvette has higher characteristics in terms of the background absorbance, it is expensive and thus cannot be easily thrown away. Further, it is only capable of reacting to certain substances (18-20). However, the polystyrene microplates are reliable and inexpensive and can be used for speeding up many different assays due to their adsorption characteristics. Although these microplates can be employed for determining the absorption in the visible light portion of the spectrum, they are not suitable for determinations in the UV range due to their relatively high background absorbance (12, 14). Obviously, selecting the microplates is not easy when measurements should be performed in the UV wavelengths instead of the visible portion of the spectrum. Since plastic polymers are applied to make disposable microplates, the background absorbance will be of importance depending on the wavelength (15).

In this study, cycloolefin microplate had higher absorption characteristics compared to polystyrene microplate. Both series of data derived from two readers illustrated that cycloolefin microplate has a lower background absorbance than the polystyrene microplate, especially in the UV range of the spectrum. Because of the consonant results of both mentioned instruments in this study, Epoch microplate spectrophotometer was selected for the remaining experiments. The absorption pattern of four different samples was then examined using Epoch microplate spectrophotometer and the obtained absorbance values of the mentioned samples all together verified the outrank of cycloolefin microplate in comparison to polystyrene microplate. Our findings are

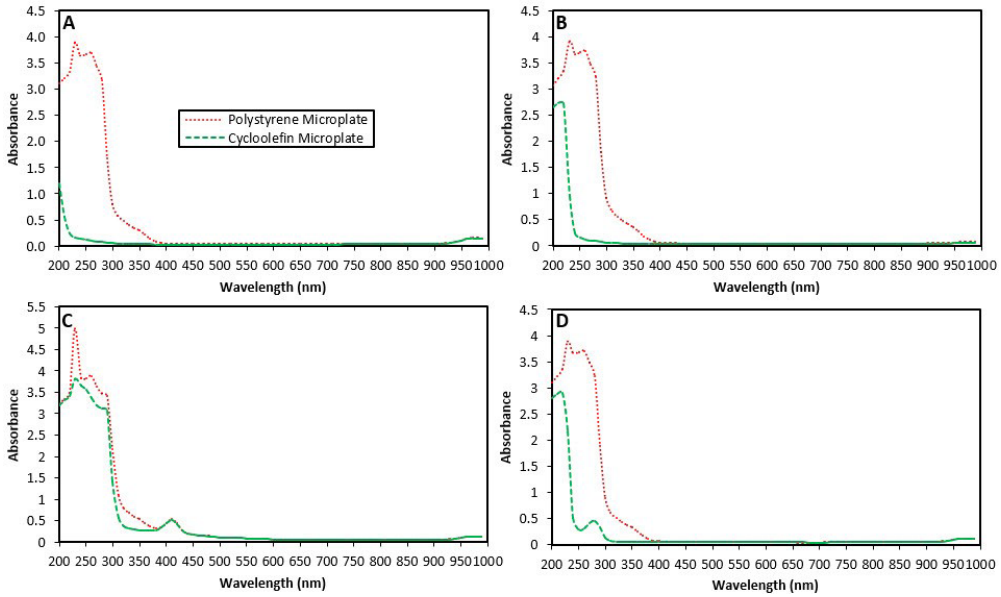

Figure 2. Spectral analysis of polystyrene and cycloolefin microplates using four different samples: (A) Distilled water, (B) L-cysteine, (C) Fetal bovine serum, and (D) tissue plasminogen activator.

in accordance with that of the study by Held in which four microplates with various constituent substances were examined for their background absorption features. He measured and compared the absorbance of distilled water containing the wells of each microplate and concluded that Costar UV plastic plates are superior to Nunc MaxiSorp, which are made of polystyrene (15).

As a conclusion, our findings showed that cycloolefin microplates are relatively transparent to UV light; therefore, they have better absorption characteristics as compared to the conventional polystyrene microplates in the UV range of the spectrum and thus are considered as a reliable choice for UV spectroscopic studies.

Conflict of interests

None.

Ethical considerations

This study as part of a project was approved by the National Research Ethics Committee with the ethics code of IR.PII.REC.1397.018 and conducted in the Pasteur Institute of Iran.

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