Label-Free Detection of Compound Aggregation Using LSPR Sensors

Investigating the aggregation behaviour of three commercial drugs

INTRODUCTION

Small molecule aggregation in aqueous media can lead to erroneous results in high-throughput screening assays and is a major concern in the pharmaceutical industry because with the pursual of false hits time and resources are wasted. Non-ionic detergents (e.g. Triton X-100) can disrupt aggregation and are therefore used frequently in screening campaigns, but they cannot fully prevent the occurrence of aggregates. Therefore, methods for their detection are important. Several methods are used for this purpose including static or dynamic light scattering, liquid chromatography (with UV or mass spectrometric detector). However, these methods lack reliability, throughput or are very complicated.

ABOUT THE TECHNIQUE

Localized surface plasmon resonance (LSPR) sensors possess several so called active areas with arrays of highly uniform metallic nano-structures. These structures exhibit specific absorption peaks due to the particles’ geometry, arrangement, size and material properties. Even very small changes of the immediate dielectric environment of the metallic nano-structures lead to detectable shifts in the plasmon resonance frequency. Therefore, binding or sedimentation of aggregates to the sensor surface can be detected with a simple absorbance measurement. LSPR AG has industrialized LSPR spectroscopy into a sensitive, versatile and powerful technique for compound aggregation detection, protein quantitation and probing biological interactions on the molecular level. The LSPR Sensors are compatible with the SBS format (see Figure 1) and can be used for high throughput screening. The dedicated software PLASMON for data collection and post-processing works with most commercial microplate readers, makes the method easy to use and yields presentable results in a short time.

MATERIALS AND EQUIPMENT

- 24datapoint LSPR Sensors (Part No. 0101-01)
- LSPR Sensor holder (Part No. 0302-01)
- Monochromator-based microplate reader
- PLASMON microplate reader control software
- 50 mM phosphate buffer (PB), pH 6.5
- Dimethyl sulfoxide (DMSO, Sigma Aldrich D5879)
- Nicardipine hydrochloride (Sigma Aldrich N7510)
- Ketoconazole (Spectrum Chemical Mfg. Corp. K1149)
- Mefenamic acid (Sigma Aldrich M4267)

SUMMARY

- Drugs with known aggregation behaviour in phosphate buffer (PB) with 1% DMSO were investigated
- Concentration series were prepared by injecting DMSO solutions into PB on LSPR Sensors
- Concentration curves with steep increases in LSPR shift due to compound aggregation were observed
- The disruptive effect of non-ionic detergent on compound aggregation could also be measured
PREPARATION OF SOLUTIONS

200 mM stock solutions in DMSO of Nicardipine hydrochloride (MW 515.99 g/mol), Ketoconazole (MW 531.43 g/mol) and Mefenamic acid (MW 241.29 g/mol) were prepared. Sample solutions in DMSO were made by diluting the stocks to achieve final assay concentrations in the range between 5 and 135 µM.

ASSAY PROCEDURE

1. 29.7 µL of PB was pipetted into each well of the LSPR Sensors and three absorbance scans of each data point were set with PLASMON and acquired with a monochromator-based microplate reader (< 30 min for 96 datapoints).
2. 0.3 µL of pure DMSO and the sample solutions were injected into three wells each to reach the desired sample concentrations and 1% DMSO in each well.
3. The sample solutions were incubated 45-60 min.
4. Another absorbance scan was set in PLASMON and measured (< 10 min for 96 datapoints).
5. Data analysis: Shifts were calculated in reference to the blank measurement and the critical aggregation concentration (CAC) was determined.

RESULTS

Figure 3 shows the LSPR shift for the dilution series of the three compounds. All three compounds show a considerable shift increase within the measured concentration range indicating aggregation (see Figure 2). Table 1 summarizes the determined CAC values. The average response value + 3σ for the two lowest concentrations (no aggregation) was chosen as critical aggregation threshold:

\[
\text{CAC} = C(R_x > 0.5(R_1 + R_2 + 3\sigma_1 + 3\sigma_2))
\]

For all three compounds, the values determined with the LSPR Sensors correspond well with the kinetic solubilities in phosphate buffer with 1% DMSO from literature (see Table 1).

![Figure 3](image_url)

**Figure 3** LSPR peak shift vs. concentration of Nicardipine (black), Ketoconazole (blue) and Mefenamic acid (green) in PB with 1% DMSO 45-60 min after injection. The graphs are offset in y direction to match their baselines. The error bars correspond to ±σ.

| Compound               | CAC (µM) | CAC (µg/ml) | kinetic solubilities (µg/ml) 
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<tr>
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<tbody>
<tr>
<td>Nicardipine hydrochloride</td>
<td>25</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>55</td>
<td>29</td>
<td>28</td>
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<tr>
<td>Mefenamic acid</td>
<td>75</td>
<td>18</td>
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**Table 1** Critical aggregation concentrations (CAC) determined from the LSPR Sensors measurements for the three compounds together with the received kinetic solubility values. [1]
To demonstrate the reproducibility of the method, the concentration series of Nicardipine used for aggregation measurements on three different LSPR Sensors. Figure 4 shows the high reproducibility of the steep increase in LSPR shift for the three measurements.

In order to demonstrate that the observed shift is indeed related to compound aggregation, the same experiment was run again with Nicardipine. This time the concentration series was not only added to wells with PB, but also to wells with PB + 0.1% Triton X-100. Triton X-100 is known to disrupt molecular aggregation. Figure 5 shows a direct comparison of the concentration series with and without the addition of Triton X-100 and a clear difference in LSPR shift indicates that the presence of Triton X-100 successfully prevented the aggregation of Nicardipine in the measured concentration range.

CONCLUSIONS

Based on the different aggregation behaviour of the three investigated model compounds (Nicardipine, Ketoconazole and Mefenamic acid) it could be shown that LSPR Sensors are a valuable addition to the spectrum of techniques to detect drug aggregates in aqueous solutions. The possibility to use them with a low cost industry standard microplate reader and the high throughput capabilities could even make the LSPR biochip the method of choice for compound aggregation detection.

REFERENCE

(1) Kinetic solubility values in µg/ml in phosphate buffer with 1% DMSO received from a major pharmaceutical company.

DISCLAIMER

The information given in this application note is given as a hint for the implementation of the LSPR biosensors only and shall not be regarded as any description of warranty of a certain functionality, condition or quality of the latter. The recipient of this application note must verify any function described herein in the real application. LSPR AG cannot be held responsible for any errors or omissions in this document. Swiss law applies.