HORIBA Scientific

Application Note

Food & Beverage

SPRi

SPR26

Antibiotic detection and inhibition assay using Surface Plasmon Resonance imaging: preliminary study for food analysis

Chloramphenicol (CAP) is a broad-spectrum antibiotic (MW 323.13 Da) that has been used in veterinary practice. However, two types of toxicity in humans induced by this small molecule have been identified. Due to these side-effects, CAP was banned from use in food-producing animals within the European community and other countries (USA and Canada, amongst others). Biosensors are a good solution for the development of rapid and low-cost screening assays in the Agri-food sector.
This application note shows that SPRi (Surface Plasmon Resonance imaging) is suitable for the development of inhibition assays for the detection of small molecules.

Materials and methods

Immobilization on the SPRi-Biochip™

A chloramphenicol derivative (CAP base) bearing a NH_2 group (Figure 1) was immobilized at 3 different concentrations (4000 µg/mL, 400 µg/mL and 40 µg/mL in 50 mM borate buffer pH 8.5) on a CS SPRi-Biochip™. Each concentration was spotted using 6 replicates.
After incubation, the spotted biochip was immersed in an ethanolamine solution to inactivate free NHS molecules, and rinsed with distilled water.

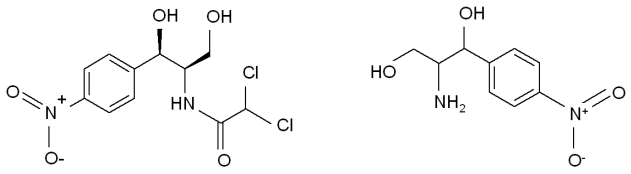

Figure 1: Structure of (left) chloramphenicol (CAP) and (right) chloramphenicol derivative (CAP base)

SPRi experiment

The aim was to detect CAP in an inhibition format. For this purpose, an antibody solution (TF22 polyclonal antibody) directed against the antibiotic was incubated with different concentrations of CAP. The CAP-antibody solution was then injected into the fluidic system and the interaction between free antibodies and immobilized CAP base was monitored by SPRi (Figure 2).

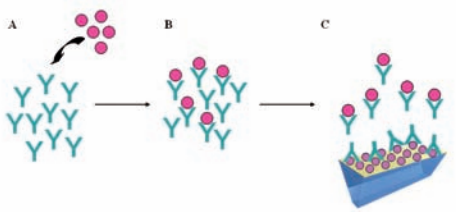

Figure 2: Principle of the inhibition assay. (A) CAP is mixed to an antibody solution, (B) some antibodies are bound specifically to CAP (C), the antibody solution is injected into the fluidic system of the SPRi system and free antibodies can bind to CAP base immobilized on the biochip

After the immobilization procedure using the SPRi-Arrayer, the spotted SPRi-Biochip™ was inserted into the SPRi-Lab+ system. The running buffer was HBS-EP and the flow rate was set to 50 µL/min. In order to build a calibration curve, eight CAP standards at different concentrations (0, 0.125, 0.25, 0.5, 1, 2.5, 5 and 10 ng/mL) were prepared. Each standard was diluted in the TF22 antibody solution (prepared at 1:180 in running buffer) and injected twice in ascending and descending order for each SPRi experiment. The biochip surface was regenerated after each sample injection using a 40 mM NaOH, 20% acetonitrile solution. The surface of the biochip was used as a negative control. Three independent SPRi experiments were run to build the calibration curve.

Results and discussion

Inhibition assay

First, in order to assess whether inhibition could be observed by SPRi, a positive (with CAP) and negative (without CAP) antibody solution was passed over the surface of the biochip. Figure 3 shows the inhibition observed for each spotting concentration after the injection of 100 ng/mL CAP mixed 1:1 with the TF22 antibody solution. Inhibition was observed for the three spotting concentrations. However, the 4000 µg/mL spotting concentration showed the highest inhibition.

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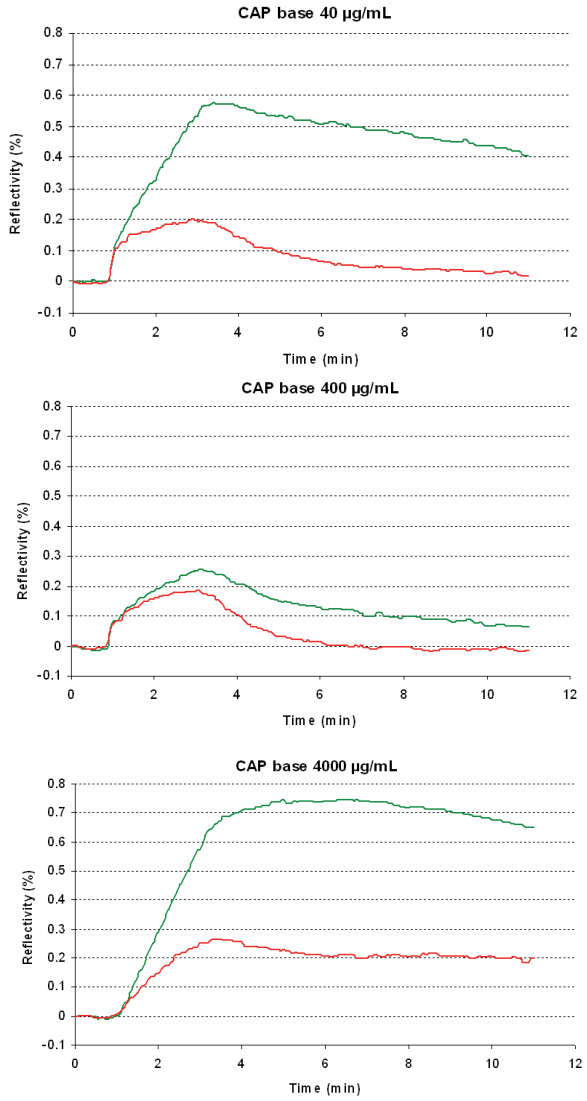

Figure 3: SPRi responses observed after the injection of the negative antibody solution (green curves) and after the injection of the positive CAP-antibody solution (red curves) for each spotting concentration.

Calibration curve

Figure 4 compares the calibration curves obtained for each CAP base spotting concentration (40 µg/mL, 400 µg/mL and 4000 µg/mL). For this purpose, the average maximum reflectivity variation obtained on each CAP base spot was plotted against the concentration of CAP in the antibody solution.

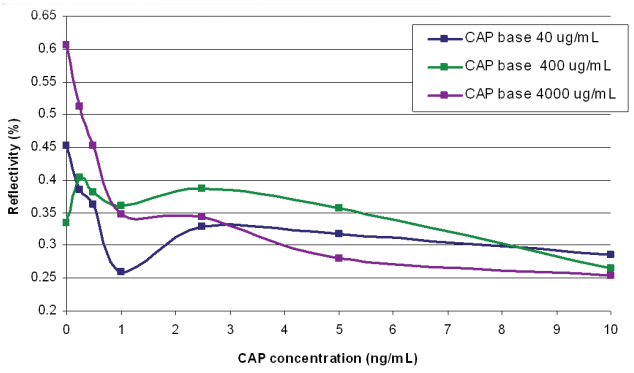

Figure 4: Calibration curve obtained using the three spotting concentrations

Thanks to the multiplex approach, it is easy to confirm that the 4000 µg/mL spotting concentration gave the widest SPRi signal range and was the most suitable spotting concentration. Then, three independent calibration curves were built using the 4000 µg/mL spotting concentration. Figure 5 shows the average curve obtained for this optimal spotting concentration. Results show good inter-assay variation, meaning that the assay format is robust and reproducible.

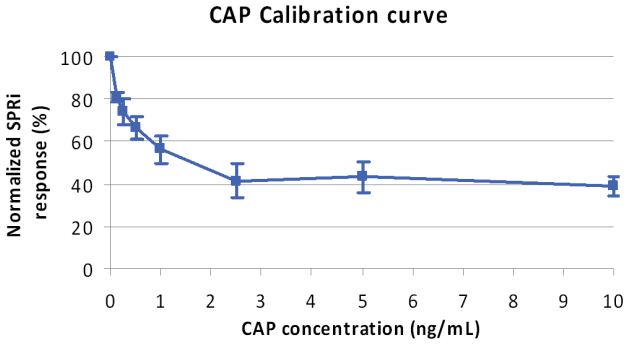

Figure 5: Average calibration curve obtained for the 4000 µg/mL spotting concentration

Conclusion

This application note shows that the HORIBA Scientific-GenOptics platform is suitable for the detection of small molecules in a direct, competitive assay. Future experiments will consist in detecting CAP in milk samples. The multiplexing capabilities of the instruments will make it possible to screen for many types of small molecule food contaminants. The ability to inject complex samples in the fluidic system will also help analyze samples from the agri-food sector.

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