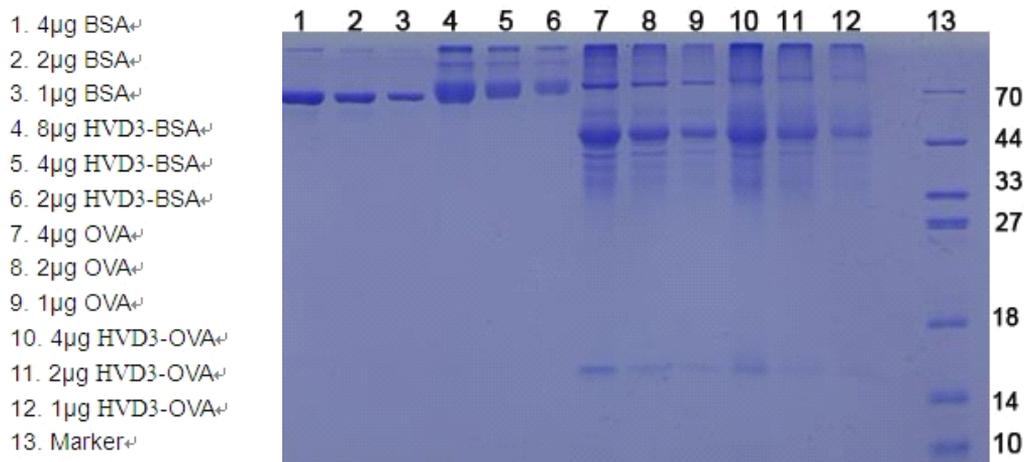


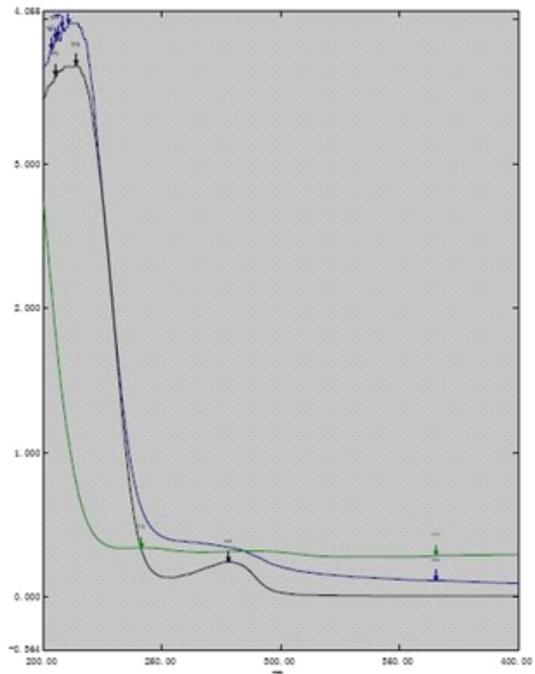
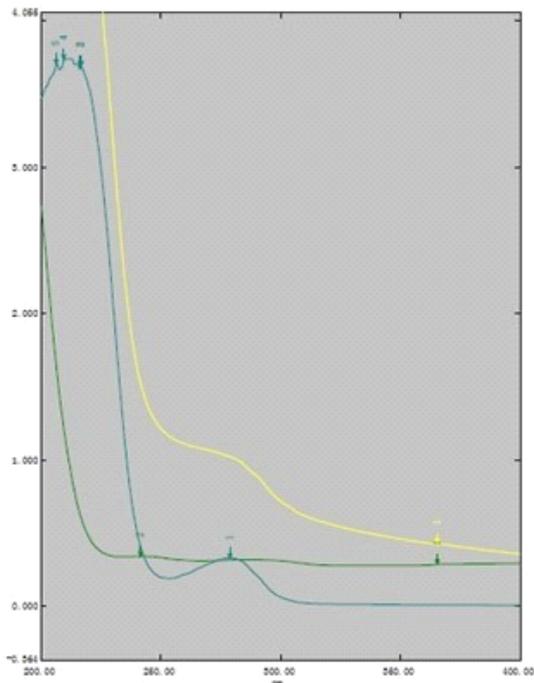
White powder, Supplied as lyophilized form in PBS, pH 7.4.



We can judge from the diagram. There are small differences the SDS-PAGE of the coupling product and carrier protein. But the strip of coupling product is dispersive. Because the small molecular have very small molecular werght (M. W.), it can been neglected comparison to the carrier protein. Meanwhile, the number of small molecules were coupled to the carrier protein is not constant, so the strips is dispersive. Therefore, 25-Hydroxyvitamin D3 and the carrier protein coupled successful.

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|--|--|
|  OVA UV-VIS curve |  BSA UV-VIS curve |
|  25-Hydroxyvitamin D3 UV-VIS curve |  25-Hydroxyvitamin D3 UV-VIS curve |
|  25-Hydroxyvitamin D3-OVA UV-VIS curve |  25-Hydroxyvitamin D3-BSA UV-VIS curve |

A

B

Picture A: From the diagram shows, the UV-VIS spectrums have obvious change between 250-280 nm about OVA, 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D3-OVA. This shows that the coupling product, which was modified by chemical process, have changed significantly. Therefore, 25-Hydroxyvitamin D3 was coupled with the carrier protein successful.

Picture B: From the diagram shows, the UV-VIS spectrums have obvious change between 250-280 nm about BSA, 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D3-BSA. This shows that the coupling product, which was modified by chemical process, have changed significantly. Therefore, 25-Hydroxyvitamin D3 was coupled with the carrier protein successful.