Journal of Pharmaceutical Analysis 14 (2024) 100983

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

journal homepage: www.elsevier.com/locate/jpa

Review paper

Advances in surface plasmon resonance for analyzing active components in traditional Chinese medicine

Jing Xie [a], Xian-Deng Li [a], Mi Li [a], Hong-Yan Zhu [a], Yan Cao [b, ***], Jian Zhang [a, **], A-Jing Xu [a, *]

[a] Faculty of Pharmacy, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, 200092, China
[b] Department of Biochemical Pharmacy, School of Pharmacy, Second Military Medical University, Shanghai, 200433, China

ARTICLE INFO

Article history:
Received 4 December 2023
Received in revised form
26 March 2024
Accepted 23 April 2024

Keywords:
Surface plasmon resonance
Traditional Chinese medicine
Optical analysis techniques
Multi-target molecular mechanism study
Biosensor

ABSTRACT

The surface plasmon resonance (SPR) biosensor technology is a novel optical analysis method for studying intermolecular interactions. Owing to in-depth research on traditional Chinese medicine (TCM) in recent years, comprehensive and specific identification of components and target interactions has become key yet difficult tasks. SPR has gradually been used to analyze the active components of TCM owing to its high sensitivity, strong exclusivity, large flux, and real-time monitoring capabilities. This review sought to briefly introduce the active components of TCM and the principle of SPR, and provide historical and new insights into the application of SPR in the analysis of the active components of TCM.

1. Introduction

Traditional Chinese medicine (TCM), a treasure of China's traditional culture, has a long history of use and extensive applications. TCM exhibits multi-component, multi-target, and multi-pathway actions. Therefore, screening and analyzing the active components of TCM are important parts of research to reveal the scientific basis for TCM use as treatments, and offer crucial guides for the discovery and development of new drugs. As the chemical components of TCM are numerous, complex, and diverse, and their contents markedly vary [1], some chemical substances have similar structures. Therefore, more accurate and efficient analytical techniques are needed. The commonly used classical methods, including chromatography, spectroscopy, and mass spectrometry [2], enable separation or identification from a qualitative or quantitative perspective, but do not allow direct exploration of the pharmacological mechanisms or dynamic observations of the pharmacological effects of the active components of TCM. Therefore, technologies with multifunctional and dynamic detection functions must be developed.

Surface plasmon resonance (SPR) biosensor, a relatively new type of sensor, applies the principle of SPR to detect and analyze target substances by combining various bioactive molecules, such as proteins, tissues, cells, and enzymes, as identification elements on biochips. SPR sensors have remarkable features, such as label-free detection, real-time monitoring, small sample size requirements, low cost, accuracy, and smooth processing [3]. As a powerful and efficient technology for studying biomolecular interactions, SPR is widely used in life sciences, environmental monitoring, food safety, and other fields [4]. SPR also has promising prospects in the field of pharmaceutical research, where this technology is extensively applied in research and discovery of drug targets, screening and verification of drug molecules, clinical immunogenicity testing, and quality control of pharmaceutical processes [5]. This review sought to provide an overview of current analysis on the active components of TCM, the principle of SPR biosensors, and the application of SPR in research and analysis of the active components of TCM. In addition, insights regarding future development of this technology are presented.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: caoyan@smmu.edu.cn (Y. Cao), zhangjian@xinhuamed.com.cn (J. Zhang), xuajing@xinhuamed.com.cn (A.-J. Xu).

https://doi.org/10.1016/j.jpha.2024.100983

2. Introduction to the active components of TCM

2.1. Overview of the active components of TCM

TCM covers a diverse range of species and is an extensive source of medical materials as it contains multiple chemical components. As advanced analytical techniques were previously lacking, the effectiveness of TCM was mainly interpreted based on traditional Chinese medical theories, with no clear understanding of the chemical substances and their pharmacological effects. Owing to this lack of understanding, TCM is perceived as pseudoscience in Western countries. To gain a better understanding of the pharmacologically active substances in TCM, studies must be conducted on its active components. The active components of TCM refer to the monomeric components that exhibit therapeutic or physiological activities and contribute to the main pharmacological effects [6]. These active components exert their pharmacological activities by interacting with biomolecules. In contrast, the non-active components of TCM refer to substances that do not exhibit clear pharmacological or therapeutic effects. These substances may play supporting roles in TCM and exhibit non-pharmacological activities.

2.2. Importance of research on the active components of TCM

TCM has been traditionally applied to treat and prevent diseases. In recent years, researchers have focused on the extraction and analysis of the active components of TCM owing to technological advancements in drug extraction and analysis, and the relatively low toxic and side effects of TCM. Evaluating the active components of TCM is of critical importance in the fields of medicine and pharmaceuticals [7].

(1) Discovery of new drugs: The active components of TCM possess unique biological activities and therapeutic effects, providing important pathways for the acquisition of active and lead compounds [8], as well as the development of drugs. By studying and understanding the active components of TCM, scientists can discover new targets and directions for drug design, thereby aiding the development of safer and more effective medications. Studies on the active components of TCM and natural drug compounds are important in the development of innovative drugs in China.
(2) Research on the pharmacological mechanisms: The active components of TCM are crucial for revealing pharmacological mechanisms. Understanding the interactions between the active components of TCM and biomolecules can help explain the pharmacological effects of TCM. In fact, scientists will gain a better understanding of the therapeutic mechanisms of TCM [9] and obtain guidelines for drug design and optimization, especially for TCM with relatively specific effects, such as Le Pill and Betel Nut. Notably, the effectiveness of the active components is more consistent with the functions of the TCM. Active components are critical for the effectiveness of TCM or a specific effect of TCM; therefore, these components can be analyzed to clarify the functions of TCM under certain conditions [10].
(3) Drug safety evaluation: Studying the active components of TCM also contributes to the evaluation of drug safety. By gaining an in-depth understanding of the interactions between the active components of TCM and biomolecules, scientists can predict adverse reactions and potential drug interactions, thereby identifying and addressing drug safety issues in advance.

Analyzing the active components of TCM not only helps us discover new active compounds but also offers other benefits, such as facilitating the scientific extraction and preparation of TCM, elucidating the mechanisms of TCM efficacy, enabling quality testing of TCM formulations, and facilitating drug screening and synthesis of new drugs.

2.3. Existing technologies commonly used in TCM research

The definition of the active components of TCM has evolved from the initial “holistic view” to the “multi-component view” [6], and is currently the “precise compound view” owing to the progress and development of analytical methods and instruments. Analysis of the active components of TCM refers to the use of modern pharmaceutical techniques to evaluate the active TCM substances extracted using appropriate methods. Once extracted, structural identification, content determination, pharmacological and toxicological properties, physicochemical properties, and biological activities can be assessed.

Currently, novel and commonly used analytical techniques include molecular imprinting [11], cell membrane chromatography [12], ultrafiltration [13], and electrochemistry [14]. Molecular imprinting uses molecularly imprinted polymers with specific recognition capabilities to analyze the active components of TCM. This method can be used to effectively extract and separate target molecules and quantitatively analyze their levels in TCM samples. By simulating the characteristics of cell membranes, cell membrane chromatography can be used to study the interactions between the active components of TCM and cell membranes, helping us understand the delivery and transport processes of TCM components within cells, thereby optimizing the pharmacological effects of TCM. The ultrafiltration method is a method that uses the selective pore size of the filter membrane to separate components in a solution, enabling selective separation of active components of different molecular sizes in TCM. By measuring electrochemical signals via electrochemistry, the electrochemical activity and reaction kinetics of the active components of TCM can be determined, ultimately enabling a deeper understanding of the chemical properties and mechanisms of the active components of TCM. However, the above methods have certain limitations. Notably, commonly used materials for analytical blotting are non-polar materials that identify hydrophobic molecules. As most of the preparation must be carried out in the organic phase, the application of this method in aqueous system is limited to the separation of water-soluble natural active substances. Sample preparation for cell membrane chromatography is difficult and easily affected by many factors, such as changes in time and the influence of activity, which may affect the results of cell membrane analysis. As the impurities retained in the membrane surface continue to accumulate during ultrafiltration, a concentration polarization phenomenon occurs when the solute concentration on the membrane surface reaches a certain limit. Owing to the generation of a gel layer, the membrane water permeability markedly decreases, thereby limiting the application of the ultrafiltration technology to a certain extent. Electrochemical sensors are very sensitive to temperature; sensors usually serves as internal temperature compensators. A stable temperature is often required during the experiment. The service life of electrochemical sensors is relatively short. Moreover, the current techniques applied to the analysis of active ingredients in TCM can only be used for a single purpose, such as separation, identification, and quantification. These techniques cannot be used to monitor interactions at the molecular level in real time, but are dynamic and label-free. Sample analysis is also limited, or active ingredients with low abundance cannot be detected. Thus, novel technologies, such as SPR, must be explored.

3. SPR biosensor

3.1. SPR phenomenon

SPR is an effective method for detecting the optical properties of substances, such as nanomaterials and biomaterials [3]. The SPR phenomenon arises from the resonance interaction between the evanescent and surface plasmon waves of a metal. When light propagates from an optically dense to an optically sparse medium, total internal reflection occurs under certain conditions. During total internal reflection, incident light propagates a certain distance in the optically sparse medium before returning to the optically dense medium [15]. A light wave entering an optically sparse medium is referred to as an evanescent wave. If a layer of a metal conductor (such as gold or silver) is added to the medium, the metal surface has numerous free valence electrons, thereby reflecting a high-energy-state gas composed of electrons, known as plasmons. Under specific conditions, the evanescent wave interacts with free electrons at the interface between the dielectric and metal film, exciting the oscillation of the surface electrons of the thin film and generating surface plasmon waves [16]. When the incident angle is at a certain value, the surface plasmon wave resonates with the evanescent wave, causing a significant decrease in the energy of the reflected light and resulting in a resonant peak on the reflectance intensity curve. This incident angle is referred to as the resonance or SPR angle. The resonance excited by a specific incident wavelength is referred to as the resonance wavelength [17]. Variations in the surface of the medium can be monitored by measuring the change in the resonance angle or resonance wavelength.

When the frequency of the incident photons matches the resonant frequency of the surface plasmons formed by free electrons on the metal film surface, the surface of the medium triggers a coherent collective oscillation of the free electrons with strong absorption or scattering peaks [18], resulting in the transfer of energy from the reflected light and the appearance of a resonance peak on the reflectance intensity curve.

3.2. Principle of SPR biosensor

Multilayered metal films are commonly used to enhance the SPR effect and improve sensitivity in practical applications of SPR [19]. The multilayered N-stack structure refers to the addition of one or more layers of dielectric materials (such as metal oxides and high-refractive-index materials) on top of a metal film. As SPR is highly sensitive to the refractive index of the medium attached to the metal film surface, changes in the properties or binding amount of the surface medium result in different resonance angles. Dynamic monitoring can be achieved by detecting changes in the SPR angle.

The design of multilayered N-stack structures can be tailored according to specific application requirements. For example, in the field of biosensing, biomolecules (such as antibodies and DNA) can be immobilized on the surface of the dielectric layer. When the analyte binds to the biofilm (recognition element) placed on the metal medium, the original SPR angle will be altered, resulting in a response that can be observed using modulation techniques [20,21]. Based on this principle, the rate of interaction and binding efficiency between biomolecules can be analyzed, enabling the identification and quantitative detection of biomolecules, and the study of molecular interactions by calculating reaction rates, dynamic equilibrium constants, and binding constants [22]. The basic working principle of SPR biosensors is shown in Fig. 1 [23]. Multilayered structures can provide a larger surface area, increasing the interaction between the sample and light, and further enhancing the detection sensitivity.

3.3. Composition and the analysis strategy of the SPR biosensor

The basic components of an SPR biosensor include an optical system, a sensing element, and a data acquisition and processing system. The sensing element, also known as the recognition element, is crucial to the SPR sensor and is mainly composed of a modified metal film and an immobilized sensitive substance, such as antibodies, cells, or enzymes. This element can convert the chemical or biological information of an analyte to change its refractive index, which can then be further processed and analyzed.

When SPR biosensors are used for detection purposes, the metal film must first be modified and then ligand molecules, such as antibodies or proteins, which can bind to the analyte, must be immobilized. According to recent studies, the use of small molecules, such as peptides or nucleic acids, can enhance the detection sensitivity. These molecules, also known as aptamers, have been proposed as alternatives to antibodies. Nucleic acid aptamers are small DNA or RNA sequences that fold into well-defined, stable, and 3D-dependent structures. Owing to these inherent characteristics, these aptamers can effectively interact with molecular targets ranging from metal ions and small organic compounds to large protein targets. Unlike protein-based antibodies and nanobodies, nucleic acid aptamers undergo reversible denaturation under adverse conditions, which increases the stability of detection [24]. Commonly used immobilization methods include coupling, chelation, and affinity adsorption [25]. The use of graphene material to encapsulate and modify the metal film can improve the sensitivity of SPR detectors [26]. However, these technologies are still being developed.

The detection strategies of SPR biosensors vary depending on factors, such as the analysis requirements, objectives, and nature of the analytes. Common detection strategies include direct, indirect, competition, and reaction kinetics methods [25], which involve immobilizing ligand molecules on the modified metal film surface and allowing the analyte to flow and bind to the surface, causing changes in parameters. This strategy is suitable for studying the binding characteristics of a single molecule and provides quantitative binding strength information. Notably, this strategy is also suitable for studying simple protein-ligand and antibody-antigen interactions. The indirect method, also known as the “sandwich” method, involves the addition of a secondary antibody after binding of the analyte to the ligand, and then detecting the interaction, which can improve the specificity and sensitivity of detection. This method is suitable for the detection of samples containing multiple targets. The competition method involves fixing the ligand and adding a solution containing the analyte and its conjugate, inducing competition for the binding sites. The SPR signal is inversely proportional to the analyte concentration. This strategy is suitable for measuring competitive binding of different substances to the same ligand. Reaction kinetics infers the kinetic parameters of the binding and dissociation of substances by monitoring the time course of their interactions. This strategy provides detailed information on the dynamics of interactions and is useful for studying complex reaction processes and dynamic changes.

Strategies are often selected according to the specific objectives of the research, properties of the substances being studied, and feasibility of the experiments. Depending on the complexity of the samples, parameters to be measured, and study objectives, an appropriate SPR detection strategy can be selected to achieve the best results.

4. Application of SPR to the analysis of the active components of TCM

Analyzing the active components of TCM is of great significance in pharmacological research, quality control of TCM, and the

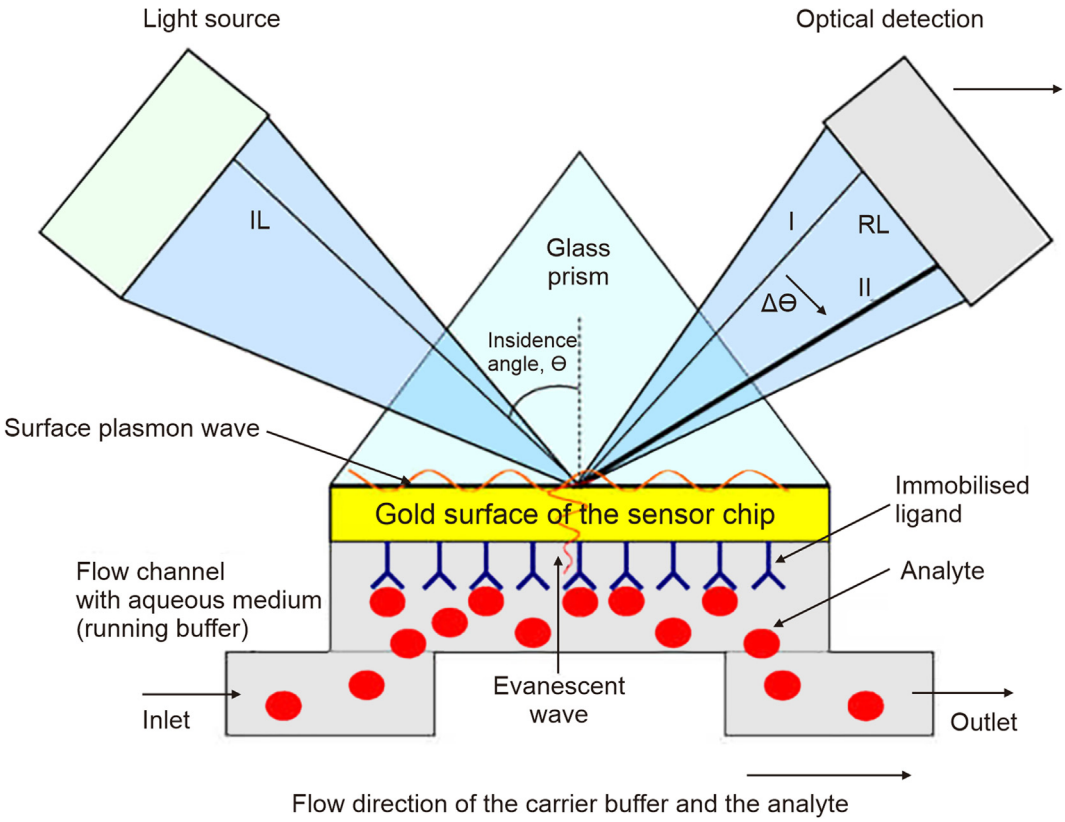

Fig. 1. Schematic of the surface plasmon resonance (SPR) phenomenon and SPR biosensor experimental rationale. (A) Incident light (IL) from a light source is irradiated at an angle (θ) onto a glass prism coated with a thin layer of gold. Part of the generated reflected light (RL) is converted into atransmitted evanescent wave. The evanescent wave transfers energy to the surface plasmons. With the change in angle, the momentum of the IL at a certain angle matches the momentum of the surface plasma, and the SPR phenomenon occurs. (B) SPR is observed as a decrease in the intensity of the reflected light at a specific angle of reflection. When the biomolecule is modified on the surface of the dielectric layer, the sample flows and binds to the biofilm placed on the metal medium (the identification element), and the original SPR angle is changed from position I to position II, resulting in a response signal. (C) One side of the gold film with the immobilised ligand (L) is integrated with microfluidic flow channel; the analyte X is passing through the channel in a continuous flow of buffer. Analyte X with affinity binds to the fixed ligand L, resulting in an increase in the proportion of the reflection angle (shift by Δθ). The dissociation of the resulting complex [XL] will result in a decrease in the reflection angle. These changes are plotted as a sensor graph (resonance unit (RU) vs. time). Reprinted with permission from Ref. [23].

development of new TCM drugs. SPR is a rapid, accurate, and label-free analytical method that can provide new measures and evaluation methods for the high-throughput screening of the active components of TCM. SPR enables studies on related target mechanisms and target kinetics and can be used to select lead compounds. Using SPR to analyze the active components of TCM enables better understanding of the efficacy and mechanism of action of TCM, which ultimately promotes the development and application of TCM.

4.1. High-throughput screening of the active components of TCM

Identifying the active components of Chinese medicines with key pharmacological activities is important for TCM studies [27] because of their extensive use to treat diseases in ancient times and modern society. SPR is characterized by high detection sensitivity, small sample size requirements, and tolerance to low purity of the test material. As a result, this technology is suitable for high-throughput analysis screening of the active components of TCM, which contains different compounds. The application process is shown in Fig. 2.

Chen et al. [28] established an SPR biosensor-based active component recognition system (SPR-AIRS) to screen the active components of herbal medicines. After validating the feasibility of the system for screening small molecules from complex drugs to derive the specificity of the protein immobilization chip, linearity, detection limit, saturation of the chip, and robustness of the system, nine active compounds with strong response signals were screened from 32 Chinese herbal medicines using this technique. These compounds include the first reported potent STAT3 ligands, neobaicalein and thujaplicin. In a subsequent study, Chen et al. [29] used the above method to screen for p38-binding actives from 34 pre-screened herbs. Subsequently, two herbs with p38 immobilization chips and high response signals, Ginkgo biloba and Artemisia, were injected into the SPR system for ligand fishing. Two active compounds were identified: isoxanthin (EPT) and ginkgolide B (GKB). The affinity constants (K_D) for EPT and GKB were 21.68 ± 2.21 and 44.71 ± 1.80 μM, respectively. The compounds significantly inhibited p38 activity and were identified as effective active components of TCM. Cao et al. [30] developed a novel SPR-based P-glycoprotein ligand screening system (SPR-PLSS) that incorporates a lentiviral particle stabilization strategy. Using this system, three active components targeting P-gp, namely magnolol (Mag), magnolol (Hon), and resveratrol (Res), were efficiently screened from an established natural product structure database containing 200 small molecule compounds. The SPR affinity test was performed using an affinity detection module. K_D values of 15.88, 65.44, and 70.01 μM were obtained for Mag, Hon, and Res, respectively. These values indicate that Mag, Hon, and Res are effective P-gp-binding ligands. Du et al. [31] screened different

Fig. 2. Application of the surface plasmon resonance (SPR) for the high-throughput screening of the active components of traditional Chinese medicine (TCM). Generated with Figdraw software (https://www.home-for-researchers.com).

active components of three TCM formulas using the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP) and employed network pharmacology, molecular docking, SPR binding, and fluorescence resonance energy transfer (FRET)-based inhibition assays for high-throughput screening of seven active components that effectively inhibited the action of the novel coronavirus 3CLpro protein. SPR analysis revealed a good interaction between epigallocatechin-3-gallate (EGCG) and 3CLpro, with a K_D of 6.17 μM. This result suggests that EGCG has a high degree of interaction with the novel coronavirus 3CLpro and high affinity.

SPR can be used for the high-throughput screening of active components of TCM and can be applied in conjunction with other research methods, such as network pharmacology, molecular docking, and FRET tests, to achieve faster, more efficient, and accurate screening of active components of TCM that have a binding effect on the target.

4.2. Target verification and mechanism of action of the active components of TCM

Chinese medicine has garnered increased global attention; however, concerns regarding its efficacy, potential side effects, and mechanisms of action persist owing to its intricate nature. These concerns may impede the broader adoption and development of TCM. To modernize Chinese medicine research and ensure its alignment with current medical and pharmacological standards, the targets of the active components should be clearly defined and the underlying molecular mechanisms should be elucidated. To uncover the targets of active substances and their mechanisms of action, SPR is of particular interest owing to its safety and specificity compared to traditional methods. Moreover, this technology is frequently combined with network pharmacological molecular docking techniques to improve research efficiency. A schematic outlining the SPR verification process of the target and mechanism of action of the active ingredients of TCM is shown in Fig. 3.

Diabetic nephropathy (DN) is a common complication of diabetes mellitus that cannot be reversed. The TCM, Gandhi capsule, improves DN symptoms, reduces urinary protein levels, and regulates lipid metabolism in podocytes in patients with DN. Zhang et al. [32] established a database containing the ingredients of the Gandhi capsule, which is commonly used in clinical practice to activate blood circulation and remove blood stasis, and a database of targets for DN. Ultra-high-performance liquid chromatography-triple quadrupole tandem mass spectrometry (UHPLC-QQQ-MS/MS) was used to identify six components in the plasma of rats orally administered the Gandhi capsule. Four protein targets, namely HNF4A, HMGCR, JAK3, and SIRT1, were screened using molecular docking analysis of kyoto encyclopedia of genes and genomes (KEGG) and Discovery Studio. The active components, baicalein and wogonoside, were verified to effectively bind to their targets using SPR. The K_D values of baicalin were 214.7, 129.2, 185.7, and 713.5 μM for SIRT1, HMGCR, JAK3, and HNF4A, respectively; and those of wogonoside were 178.7, 4.523, 4.072, and 0.5545 μM for SIRT1, HMGCR, JAK3, and HNF4A, respectively. By corroborating these findings using protein blotting experiments, the investigators identified the molecular mechanisms by which baicalein and wogonoside mitigate the adverse effects of high glucose concentrations on cell viability and podocyte protein expression. Furthermore, the activation of *p*-V-akt murine thymoma viral oncogene homolog (*p*-AKT), p-phosphoinositide 3-kinase (p-PI3K), and phosphorylation-AMP-activated protein kinase (p-AMPK) was found to increase. Luo et al. [33] used an ultra-high-pressure liquid chromatography withlinear ion trap-Orbitrap tandem mass spectrometer (UHPLC-LTQ-Orbitrap) system to rapidly identify 53 major compounds in Solid Cough Soup. After virtual screening via

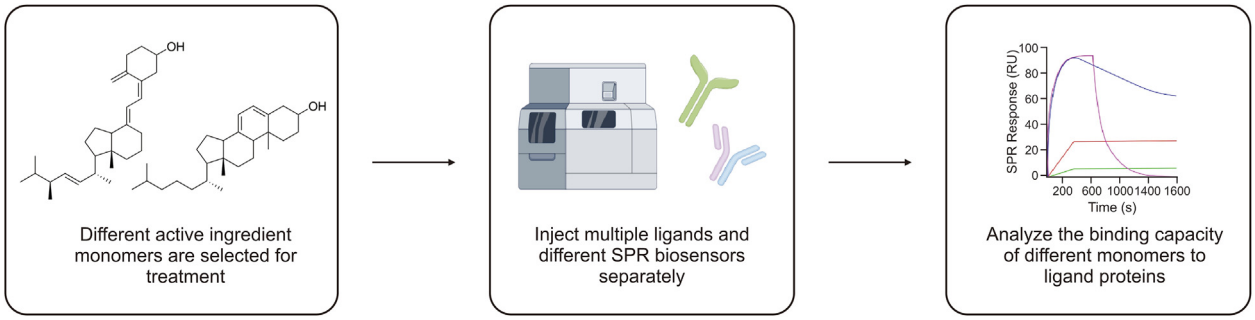

Fig. 3. Schematic of the process of the surface plasmon resonance (SPR) verification of the target, and mechanism of action of the active ingredients of traditional Chinese medicine (TCM). Generated with Figdraw software (https://www.home-for-researchers.com).

network pharmacology, the tumor necrosis factor (TNF) signaling pathway with 13 key targets was predicted. Four target proteins, namely nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NFKB1), matrix metalloproteinase-9 (MMP-9), TNF-α, and mitogen-activated protein kinase 1 (MAPK1), were selected and immobilized on a microarray using the standard amino-coupling method for SPR analysis. The K_D value of mangosteen and NFKB1 was 32.5 μM; that of isoglycyrrhizin and TNF-α was 3.6 μM; that of isoglycyrrhizin and MMP-9 was 2.7 μM; and that of tonic dihydroflavonoid and MAPK1 was 6.05 μM. Accordingly, new relevant targets of the active components were identified to determine the mechanism of the anticrucial effects of Solidago Cough and Asthma Soup.

Xu et al. [34] used FRET to screen the active components of tiger nuts, specifically tiger nut glycosides and resveratrol, to determine their potential to inhibit the hydrolytic activation of the 3CLpro and Plpro proteins. Using SPR, these investigators verified that both tiger nut glycosides and resveratrol exhibited high affinity for the 3CLpro and PLpro proteins of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), SARS-CoV, and Middle East respiratory syndrome coronavirus (MERS-CoV). In addition, thujaplicin displayed strong dose-dependent binding to the 3CLPro and PLPro proteins of these viruses, with K_D values of 16.01, 5.428, 16.44, 39.53, 1.976, and 7.745 μM. Resveratrol also bound to these proteins in a dose-dependent manner with K_D values of 0.9812, 92.51, 7.312, 12.35, 1.555, and 6.1 μM. Based on the SPR results, thujaplicins and resveratrol target the 3Clpro and PLpro proteins. Radix Paeonia lactiflora (RPA) exhibits anti-inflammatory and immunomodulatory effects and has traditionally been used to treat rheumatoid arthritis and hepatitis. However, little is known about the anti-inflammatory mechanism of RPA, and the potential bioactive components and direct binding target proteins that contribute to its pharmacological activities. Lv et al. [35] used an SPR screening system to identify the active components of RPA, paeoniflorin and salvinorin, which bind to TNF receptor-1 (TNF-R1). The affinity constants of these components were determined using SPR. The K_D values of paeoniflorin and salvinorin were 4.9 and 11.8 μM, respectively, confirming that paeoniflorin and salvinorin in RPA can play important roles by directly binding to TNF-R1. This result also indicates that TNF-R1 is the target of action of the active ingredient in Paeonia lactiflora. Zhang et al. [36] found that rutaecarpine (RUT), the active ingredient of the TCM, Cornus officinalis, could ameliorate dextran sodium sulfate-induced colitis by activating nuclear factor erythroid 2-related factor 2 (NRF2). SPR confirmed that RUT could bind to its inhibitory protein, KEAP1, with a K_D of 19.6 μM. Further studies revealed that RUT promotes the release of NRF2 by acting on the target KEAP1 protein, which in turn induces the nuclear translocation of NRF2 and downstream antioxidant reactions. Li et al. [37] explored the effects and potential molecular mechanisms of berberine (Ber) in gastric cancer (GC) and assessed the inhibitory activity and binding affinity of Ber to the bromodomain-containing protein 4 (BRD4) protein using homogeneous time resolved fluorescence (HTRF) and SPR. Ber was found to bind to the BRD4 protein in a dose-dependent manner and inhibit BRD4 protein activity with a K_D value of 10 μM. The quantitative polymerase chain reaction (qPCR) results further confirmed that Ber effectively downregulated the mRNA levels of the BRD4 transcriptional targets (e.g., c-MYC, BCL2, and BCL6), suggesting that Ber is a novel BRD4 inhibitor whose anti-GC effect is mainly mediated by BRD4 inhibition.

In conclusion, understanding the targets and mechanisms of action of the active components of TCM is pivotal for research in this field. Notably, SPR has emerged as a powerful tool for this endeavor, enabling detailed analysis of complex mechanisms and precise identification of targets and their associated mechanisms of action.

4.3. Binding kinetics of the active components of TCM

To comprehensively assess the pharmacological activity and efficacy of the active components of TCM and elucidate the *in vitro* inhibitory activity of drug candidates on their possible pharmacological effects *in vivo*, the drug-target binding kinetic process should not be ignored when evaluating the affinity of the active ingredient to the target. Over the past decade, a direct correlation has been found between *in vivo* efficacy and drug-target binding kinetics. As a result, research efforts are increasingly focused on this area and its integration into drug development and applications [38]. The real-time monitoring capabilities of SPR biosensors render them particularly suitable for studying the binding kinetics of these ingredients to the target proteins.

Pan et al. [39] used SPR to monitor the binding process of α-glucosidase to three polyphenol compounds (catechin gallate, catechin gallate and gallocatechin gallate) in green tea in real time, and determine the binding kinetic parameters between the compounds and target proteins. By combining catechin gallate with the prediction model of the drug-α-glucosidase binding kinetics established previously by the group, the dynamic process of drug-target binding kinetics with α-glucosidase during different administration processes could be assessed. Li et al. [40] employed ultra performance liquid chromatography coupled with quadrupole exactive mass spectrometry (UPLC-Q Exactive MS), methods for identifying reference standards, and *in vitro* enzyme kinetics experiments to identify potential uric acid-lowering components in chrysanthemums. SPR was used to determine the binding kinetic parameters of potential inhibitors (diosmetin, luteolin, and apigenin) in chrysanthemum with xanthine oxidase. The association rate constants (K_{on}) were 1.26×10^6, 5.23×10^5, and 6.36×10^5 mol·L^{-1}·s^{-1}; and the dissociation rate constants (K_{off}) were 10.93×10^{-2}, 1.59×10^{-2}, and 5.3×10^{-2} s^{-1}, respectively. Finally, a validated mathematical model of xanthine oxidase target occupancy was used to evaluate the binding kinetics of the inhibitors with xanthine oxidase *in vivo*. In this study, the *in vivo* target occupancy processes of the three active components in chrysanthemum administered via different routes were investigated using ultra performance liquid chromatography-mass spectrometry (UPLC-MS), concentration-response methods, SPR, and the xanthine oxidase target occupancy model, thereby providing a novel research approach for studying the pharmacokinetics of the active components of TCM and their binding kinetics with drug targets. Tsai et al. [41] conducted an initial analysis using computer-generated pharmacophore models. The binding affinities of quercetin and kaempferol to human glutamate cysteine ligase were verified using SPR. Based on the binding kinetics results, quercetin and kaempferol rapidly bound to and slowly dissociated from human glutamate cysteine ligase at different concentrations.

In summary, SPR enables real-time dynamic monitoring and the study of drug-target binding kinetics of the active components of TCM by simulating the drug action environment. Overall, this study provided new insights into the *in vivo* effects of active components of TCM. The workflow for studying the target-binding kinetics of active components of Chinese herbal medicines using SPR is summarized in Fig. 4.

4.4. Identification of the active components of TCM as lead compounds

TCM is a crucial tool for identifying new drug candidates. SPR enables real-time, precise, and reliable interaction analysis between molecules and serves as a new approach for drug discovery. By combining SPR with TCM molecular libraries, the limitations of conventional drug-screening techniques can be overcome. This

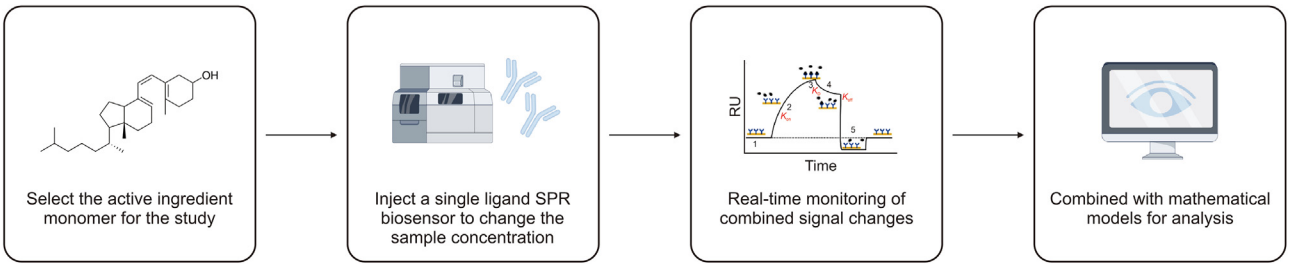

Fig. 4. The surface plasmon resonance (SPR) for the study of the targeted binding kinetics of the active components of traditional Chinese medicine (TCM). Generated with Figdraw software (https://www.home-for-researchers.com).

harmonious melding minimizes the arbitrariness and workload of the screening process, distinguishing optimal active components as lead compounds and ultimately enhancing the efficiency and success rate of drug development.

Wang et al. [42] used SPR to screen potential epidermal growth factor receptor (EGFR) inhibitors from four kaempfer quinone active components, namely levamonin, acetyl comfrey, β,β-dimethylacryloyl comfrey, and β-acetoxyisovaleryl comfrey, in Xinjiang comfrey. Among the four monomers, only β,β-dimethylacryloyl viologen had a significant binding effect with EGFR and good affinity. The other three monomers did not exhibit a clear response, which indicated that β,β-dimethylacryloyl viologen might be an inhibitor of EGFR. Therefore, β,β-dimethylacryloyl viologen could be selected as a lead compound, and its structural composition could be optimized to serve as an inhibitor of EGFR. Su et al. [43] screened a small-molecule compound, apigenin, which can bind to signal transducer and activator of transcription 3 (STAT3) with high specificity, from 50 monomers of TCM using SPR and confirmed its inhibitory effect on STAT3 through experiments. Based on the results, apigenin can be used as a lead compound in the study of anticancer drugs, which provides a theoretical basis for the anticancer effects of apigenin. He et al. [44] screened 17 compounds with potential 14-3-3τ protein binding activity from 82 alternative natural products. By carrying out SPR validation, the investigators found that 10 of the compounds exhibited high affinity to the 14-3-3τ protein. Combined with structural analysis and optimization, this SPR result highlighted a new method for the discovery of antitumor lead compounds from natural products. Wang et al. [45] also studied the effects of ginseng. Nine saponins were identified using liquid-liquid mass spectrometry, and three ginsenosides with programmed cell death protein 1 (PD-1)-binding activity were screened using SPR, including ginsenoside Rg1, ginsenoside Rb1, and ginsenoside Re. Competitive inhibition between the active substances and PD-1/programmed cell death ligand 1 (PD-L1) was detected using SPR. Of note, ginsenosides Rb1 and Re were not found to exhibit inhibitory activity. However, ginsenoside Rg1 inhibited the binding of PD-1/PD-L1 in a dose-dependent manner. As the concentration of the small molecules increased, the signal value decreased continuously, and the maximum inhibition rate reached 52.56%. Thus, ginsenoside Rg1 specifically inhibits the interaction between PD-1 and PD-L1. Ginsenoside Rb1 and ginsenoside Re exerted dose-dependent inhibition; however, the maximum inhibition rate was less than 50% and the inhibition efficiency was lower than that of ginsenoside Rg1, which proved that ginsenoside Rg1 could be used as a lead compound to inhibit immune signaling pathways.

In summary, SPR can be applied not only to high-throughput screening of the active components of TCM but also to the identification of optimal structural components that strongly bind to disease proteins from complex and diverse active components. This study provides a new avenue for the treatment of various diseases. The SPR approach used to select lead compounds from the active components of TCM is summarized in Fig. 5.

5. Summary and outlook

5.1. Advantages of SPR for analyzing the active components of TCM

SPR offers distinctive benefits in research on the active components of TCM. (1) Distinct specificity: Based on the working principle of SPR, only the interacting molecules can bind to each other and be detected. Owing to the complex composition of TCM, SPR can be used to accurately analyze the active substances. (2) High sensitivity: SPR biosensors are highly sensitive instruments with low detection limits [5]. The low content of the active components of TCM can be comprehensively detected and analyzed. Compared to the traditional fluorescent labelling detection of ground content components, SPR can reduce sample contamination and loss without the need for labelling. Thus, SPR is a suitable method for examining valuable samples. (3) Real-time monitoring: The signal response value of SPR biosensors is related to the quality of the bound molecules on the chip surface. As a result, real-time monitoring of the bound active components can be performed and the dynamic environment in the body can be simulated, thereby mimicking an analysis closer to that performed to obtain real data. (4) High-throughput SPR biosensors have a high tolerance

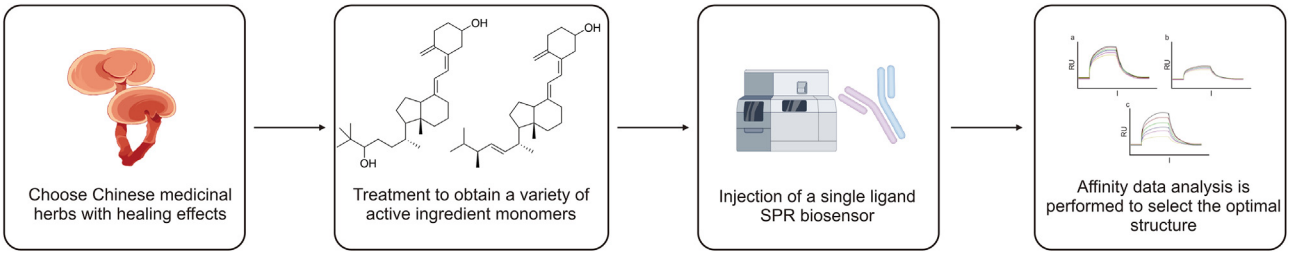

Fig. 5. The surface plasmon resonance (SPR) method for identifying active ingredients as lead compounds in traditional Chinese medicine (TCM). Generated with Figdraw software (https://www.home-for-researchers.com).

in terms of sample purity. The extraction of TCM can be performed without a complex purification process, which markedly improves the research efficiency and enables high-throughput screening [17].

5.2. Limitations and countermeasures of SPR for analyzing the active components of TCM

Despite their numerous advantages, SPR biosensors also have limitations. Researchers have actively developed new strategies to promote the application of SPR for research and analysis.

5.2.1. Instrumental factors

Sample detection using SPR normally requires relatively large and expensive instruments, which depends on the availability of laboratories and sample locations. However, in recent years, remarkable efforts have been dedicated to the development of portable SPR instruments. For example, Affinite Instruments has developed a portable SPR device called P4SPR, which offers multi-channel analysis with compact size and portability. This device has been widely used in outdoor field experiments on pesticide residues, trace drugs, pollutants, explosives, and other environmental tests [46]. Plasmetrix, a Canadian company, introduced a high-precision SPR instrument that is a personal and portable desktop called Corgi IIF. This device is only 15 cm in length, has configurable performance, and facilitates the study of molecular and protein functions [47]. Owing to the special optical waveguide characteristics of the photonic crystal fiber (PCF) structure, this device can transmit light signals with multiple wavelengths. As a result, PCF achieves multifunctional integration in a compact single-optical structure. PCF also offers high sensitivity and flexibility. Different sensor characteristics and application requirements can be achieved by adjusting the shapes and sizes of the core or air-filled holes. This flexibility makes it possible to customize designs based on specific sensing requirements, thereby simplifying sensor manufacturing and leading to the development of various portable SPR instruments [48–50]. Research groups have attempted to transform or connect SPR sensors to smartphones, such as handheld SPR instruments, USB interface SPR instruments, and SPR instruments based on microfluidic technologies [51,52]. The stability and practicality of these types of sensors are actively being investigated. The development of portable SPR sensors provides a wide selection of experimental locations with improved sensitivity for certain applications. However, their performance is limited by their compact size, unlike standard devices that have more options for control and adjustment, enabling precise control of measurement conditions and parameters.

5.2.2. Biosensor factors

The use of SPR instruments normally requires special biosensor chips and surface modification of the medium, which may go beyond the life sciences knowledge of practitioners. Previously, the production of biosensor chips often involved the immobilization of different ligands on a metal medium surface. Commonly used immobilization techniques include covalent immobilization, non-covalent immobilization, etc. The most popular covalent coupling groups are the amine, thiol, aldehyde, and carboxyl groups. Covalent immobilization methods are suitable for studying various biomacromolecular complexes, such as proteins, lipids, and DNA. For example, lipid bilayers are often combined with thiols. Antibodies and aptamers are commonly targeted using non-covalent methods. However, under certain situations, the bound target molecules are too far away from the sensing surface. Cooperation between engineering and material science disciplines is recommended to standardize the production of biosensor chips for SPR sensors. For example, material science can contribute to the research and optimization of materials used to fabricate biosensor chips to achieve enhanced sensitivity and stability [18–20]. On the other hand, engineering offers micro- and nano-techniques, such as lithography, wet-etching, and thin-film deposition, for the manufacturing of sensor structures with miniature dimensions [53]. Transcriptomics can be employed to create 3D ligands to improve the reaction specificity. The application of SPR sensors is a multidisciplinary endeavor that requires theoretical knowledge and more stable and reliable models for the analysis of the active components of TCM.

5.2.3. Technology expansion factors

SPR has certain limitations. In particular, this technology can only screen active components and reveal their binding characteristics with target proteins. SPR cannot be used to directly determine the chemical nature of active components or assess their biological effects. Therefore, the use of SPR as a stand-alone application is limited when a comprehensive analysis of the active components of TCM is required. SPR is typically used in conjunction with other techniques and methods for these applications. Currently, SPR is commonly used with spectroscopy, mass spectrometry, computer molecular docking, network pharmacology, etc. [17]. In addition, emerging integrated approaches include the combination of SPR with surface-enhanced Raman scattering (SERS) to achieve higher sensitivity and resolution [17]. SPR combined with microarray technology can be used for the high-throughput analysis of multiple samples. Different molecular targets immobilized on the microarray surface of an SPR chip can be used to monitor SPR signal changes caused by cell binding or molecular interactions, enabling high-throughput analysis of multiple samples and targets [54]. SPR can also be combined with biological labeling techniques, such as fluorescence labeling and enzyme labeling, to enhance the quantitative and qualitative detection of molecular signals, thereby enabling specific detection of biological molecules. This combination can also achieve real-time monitoring and visualization of the binding between the active components of TCM and targets, as well as multi-labeling and high-spatial-resolution targeting [17]. In recent years, single-cell and spatial transcriptomics have gained attention as high-throughput and highly specific techniques. These techniques may be combined with SPR. Using fluorescence labeling for the screened active components, the changes in fluorescence signals can be monitored in real time using SPR, including changes in concentration. By combining single-cell and spatial transcriptomics, we can gain insights into the spatial and specific mechanisms of action of active components in cells and tissues, providing suggestions for studying the distribution and metabolic dynamics of active components *in vivo*. However, specific experimental combination methods require further investigation.

5.3. Prospects and outlook of SPR for analysis of the active components of TCM

TCM formulas are complicated. Studies have suggested that TCM functions through multiple targets and components. Exploring the bioactive substances of TCM based on their characteristics is a key hotspot in TCM research, and a bottleneck problem that must be resolved to advance the TCM industry in China. SPR offers multiple advantages and is suitable for analyzing the active components of TCM. In recent years, new technologies, such as SPR imaging and SPR microscopy [55,56], have emerged, which can overcome the current limitations of SPR. The application of SPR for the analysis of the active components of TCM will lead to higher throughput, higher efficiency, and integration of multiple technologies. Moreover, such application is an interdisciplinary collaboration involving not only knowledge of TCM but also material science, engineering, statistics, disease diagnosis, and other multidisciplinary theories and concepts. Through multidisciplinary cooperation, new application areas can be discovered, enabling more efficient completion of research tasks in the

analysis of the active components of TCM and identification of new paths for the modernization of TCM research.

CRediT authorship contribution statement

Jing Xie: Data curation, Investigation, Writing – original draft, Writing – review & editing. **Xian-Deng Li:** Investigation, Writing – review & editing. **Mi Li:** Investigation, Writing – review & editing. **Hong-Yan Zhu:** Data curation, Investigation. **Yan Cao:** Supervision, Writing – review & editing. **Jian Zhang:** Funding acquisition, Resources, Supervision, Writing – review & editing. **A-Jing Xu:** Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (Grant No.: 82072142), the National Key R&D Program of China (Grant No.: 2020YFC2005502) and the Science and Technology Commission of Shanghai Municipality (Grant No.: 19401900500).

References

[1]] J. Liu, H. Xiao, Research progress on pharmacodynamic substances of Chinese Medicine based on chromatographic techniques, Acta Pharm. Sin. 54 (2019) 73–81.
[2] C. Liu, Z. Zuo, F. Xu, et al., Authentication of herbal medicines based on modern analytical technology combined with chemometrics approach: A review, Crit. Rev. Anal. Chem. 53 (2023) 1393–1418.
[3] V. Yesudasu, H.S. Pradhan, R.J. Pandya, Recent progress in surface plasmon resonance based sensors: A comprehensive review, Heliyon 7 (2021), e06321.
[4] C. Luo, Y. Wang, W. Huang, et al., A miRNA biosensor based on SPR technology and its application in tumor detection, Int. J. Lab. Med. 4 (2015) 525–527.
[5] J. Wang, Q. Wang, S. Song, Research progress of surface plasmon resonance technology in drug discovery, J. Chin. Pharm. Sci. 29 (2020) 504–513.
[6] B. Wang, Q. Zhou, Understanding of the active ingredients of traditional Chinese medicine and its research methods, Zhongguo Zhong Yao Za Zhi 26 (2001) 10–13.
[7] C. Wang, X. Bai, C. Wang, Traditional Chinese medicine: A treasured natural resource of anticancer drug research and development, Am. J. Chin. Med. 42 (2014) 543–559.
[8] Q. Wang, W. Li, H. Hu, et al., Monomeric compounds from traditional Chinese medicine: New hopes for drug discovery in pulmonary fibrosis, Biomed. Pharmacother. 159 (2023), 114226.
[9] S. Wang, J. Fu, H. Hao, et al., Metabolic reprogramming by traditional Chinese medicine and its role in effective cancer therapy, Pharmacol. Res. 170 (2021), 105728.
[10] Z. Hu, Analysis of the active ingredients and efficacy of traditional Chinese medicine, Inf. Tradit. Chin. Med. 15 (1998), 22.
[11] S. Li, Y. Chang, F. Cheng, et al., Multi-template molecularly imprinted solid phase extraction and its application in the extraction and separation of multi-components from traditional Chinese medicine, Acta Pharm. Sin. 56 (2021) 751–760.
[12] D. Tang, W. Xiao, Z. Qian, et al., Rapid screening of potential analgesic ingredients from Draconis Resina by live cell immobilized chromatography coupled with HPLC-DAD-TOF-MS, Chin. Tradit. Herb. Drugs 50 (2019) 2539–2544.
[13] S. Wu, H. Yang, P. Li, Application of the affinity ultrafiltration coupled with LC-MS technology in screening active components of traditional Chinese medicines, Yao Xue Xue Bao 51 (2016) 1060–1067.
[14] J. Xie, Y. Fu, J. Jin, et al., Determination of rutin in the zhenjujiangya tablet by electrochemical method, Chem. Sens. 36 (2016) 58–62.
[15] A. Abbas, M.J. Linman, Q. Cheng, New trends in instrumental design for surface plasmon resonance-based biosensors, Biosens. Bioelectron. 26 (2011) 1815–1824.
[16] J. Cao, J. Yang, L. Zhao, et al., Graphene oxide@gold nanorods-based multiple-assisted electrochemiluminescence signal amplification strategy for sensitive detection of prostate specific antigen, Biosens. Bioelectron. 99 (2018) 92–98.
[17] T. Chen, J. Xin, S.J. Chang, et al., Surface plasmon resonance (SPR) combined technology: A powerful tool for investigating interface phenomena, Adv. Mater. Interfaces 10 (2023), 2202202.
[18] R. Singh, Z. Wang, C. Marques, et al., Alanine aminotransferase detection using TIT assisted four tapered fiber structure-based LSPR sensor: From healthcare to marine life, Biosens. Bioelectron. 236 (2023), 115424.
[19] B. Kaur, S. Kumar, B.K. Kaushik, 2D materials-based fiber optic SPR biosensor for cancer detection at 1550 nm, IEEE Sens. J. 21 (2021) 23957–23964.
[20] P.S. Pandey, S.K. Raghuwanshi, S. Kumar, Recent advances in two-dimensional materials-based kretschmann configuration for SPR sensors: A review, IEEE Sens. J. 22 (2022) 1069–1080.
[21] P.S. Pandey, S.K. Raghuwanshi, A. Shadab, et al., SPR based biosensing chip for COVID-19 diagnosis-a review, IEEE Sens. J. 22 (2022) 13800–13810.
[22] A. Olaru, C. Bala, N. Jaffrezic-Renault, et al., Surface plasmon resonance (SPR) biosensors in pharmaceutical analysis, Crit. Rev. Anal. Chem. 45 (2015) 97–105.
[23] G. Safina, Application of surface plasmon resonance for the detection of carbohydrates, glycoconjugates, and measurement of the carbohydrate-specific interactions: A comparison with conventional analytical techniques. A critical review, Anal. Chim. Acta 712 (2012) 9–29.
[24] C.C. Chang, Recent advancements in aptamer-based surface plasmon resonance biosensing strategies, Biosensors 11 (2021), 233.
[25] M. Wang, X. Duan, H. Shao, et al., Application prospect of surface plasmon resonance technique in quality control of biopharmaceutical products, J. China Pharm. Univ. 23 (2020) 2257–2260.
[26] W. Wei, J. Nong, Y. Mei, et al., Single-layer graphene-coated gold chip for enhanced SPR imaging immunoassay, Sens. Actuat. B Chem. 273 (2018) 1548–1555.
[27] Q. Jiao, R. Wang, Y. Jiang, et al., Study on the interaction between active components from traditional Chinese medicine and plasma proteins, Chem. Cent. J. 12 (2018), 48.
[28] L. Chen, D. Lv, X. Chen, et al., Biosensor-based active ingredients recognition system for screening STAT3 ligands from medical herbs, Anal. Chem. 90 (2018) 8936–8945.
[29] L. Chen, D. Wang, D. Lv, et al., Identification of eupatilin and ginkgolide B as p38 ligands from medicinal herbs by surface plasmon resonance biosensor-based active ingredients recognition system, J. Pharm. Biomed. Anal. 171 (2019) 35–42.
[30] Y. Cao, Y. Cao, Y. Shi, et al., Surface plasmon resonance biosensor combined with lentiviral particle stabilization strategy for rapid and specific screening of P-Glycoprotein ligands, Anal. Bioanal. Chem. 413 (2021) 2021–2031.
[31] A. Du, R. Zheng, C. Disoma, et al., Epigallocatechin-3-gallate, an active ingredient of Traditional Chinese Medicines, inhibits the 3CLpro activity of SARS-CoV-2, Int. J. Biol. Macromol. 176 (2021) 1–12.
[32] Q. Zhang, Q. Ye, X. Huang, et al., Revealing active components, action targets and molecular mechanism of Gandi capsule for treating diabetic nephropathy based on network pharmacology strategy, BMC Complement. Med. Ther. 20 (2020), 362.
[33] Z. Luo, G. Yu, W. Wang, et al., Integrated systems pharmacology and surface plasmon resonance approaches to reveal the synergistic effect of multiple components of gu-Ben-ke-Chuan decoction on chronic bronchitis, J. Inflamm. Res. 14 (2021) 1455–1471.
[34] H. Xu, J. Li, S. Song, et al., Effective inhibition of coronavirus replication by *Polygonum cuspidatum*, Front. Biosci. (Landmark Ed.) 26 (2021) 789–798.
[35] D. Lv, J. Xu, M. Qi, et al., A strategy of screening and binding analysis of bioactive components from traditional Chinese medicine based on surface plasmon resonance biosensor, J. Pharm. Anal. 12 (2022) 500–508.
[36] Y. Zhang, T. Yan, D. Sun, et al., Rutaecarpine inhibits KEAP1-NRF2 interaction to activate NRF2 and ameliorate dextran sulfate sodium-induced colitis, Free. Radic. Biol. Med. 148 (2020) 33–41.
[37] H. Li, K. Luo, Z. Yang, et al., Berbamine suppresses the growth of gastric cancer cells by inactivating the BRD4/c-MYC signaling pathway, Drug Des. Devel. Ther. 16 (2022) 129–141.
[38] R.A. Copeland, Evolution of the drug-target residence time model, Expert Opin. Drug Discov. 16 (2021) 1441–1451.
[39] F.L. Pan, et al., Study on binding kinetics profiles of tea polyphenols-α-glucosidase interaction, Zhongguo Zhong Yao Za Zhi 45 (2020) 4472–4481.
[40] X. Li, Y. Liu, F. Liu, et al., Study on drug-target binding kinetics profiles of flavonoids in *Chrysanthemum morifolium* and xanthine oxidase, Zhongguo Zhong Yao Za Zhi 46 (2021) 1822–1831.
[41] K.C. Tsai, Y. Zhang, H.Y. Kao, et al., Pharmacophore-driven identification of human glutaminyl cyclase inhibitors from foods, plants and herbs unveils the bioactive property and potential of Azaleatin in the treatment of Alzheimer's disease, Food Funct 13 (2022) 12632–12647.
[42] M. Wang, S. Li, X. Dong, et al., Interactions between EGFR and four compounds from Arnebia euchroma based on SPR, Chin. J. Exp. Tradit. Med. Formulae 24 (2018) 32–36.
[43] X. Su, H. Zhang, N. Zhang, et al., Screening small molecular inhibitors of STAT3 based on surface plasmon resonance technology, J. Pharm. Pract. 39 (2021) 515–519.
[44] T. He, W. Jia, W. Wang, et al., Screening of 14-3-3τ protein inhibitors from natural products based on fluorescence spectroscopy, surface plasmon resonance and molecular docking technique, J. Int. Pharm. Res. 46 (2019) 582–590.

[45] D. Wang, P. Tu, Y. Huang, et al., Identification of PD-1 small molecule inhibitors and validation in Panax ginseng, Acta Pharm. Sin. 55 (2020) 2428−2434.
[46] T. Brulé, G. Granger, N. Bukar, et al., A field-deployed surface plasmon resonance (SPR) sensor for RDX quantification in environmental waters, Analyst 142 (2017) 2161−2168.
[47] J. Yang, X. Lin, N. Xing, et al., Structure-based discovery of novel nonpeptide inhibitors targeting SARS-CoV-2 m^{pro}, J. Chem. Inf. Model. 61 (2021) 3917−3926.
[48] S. Jain, K. Choudhary, S. Kumar, Photonic crystal fiber-based SPR sensor for broad range of refractive index sensing applications, Opt. Fiber Technol. 73 (2022), 103030.
[49] B. Li, T. Cheng, J. Chen, et al., Graphene-enhanced surface plasmon resonance liquid refractive index sensor based on photonic crystal fiber, Sensors 19 (2019), 3666.
[50] Q. Liu, J. Sun, Y. Sun, et al., Surface plasmon resonance sensor based on photonic crystal fiber with indium tin oxide film, Opt. Mater. 102 (2020), 109800.
[51] Y. Huang, L. Zhang, H. Zhang, et al., Development of a portable SPR sensor for nucleic acid detection, Micromachines 11 (2020), 526.
[52] G.P. Singh, N. Sardana, Smartphone-based surface plasmon resonance sensors: A review, Plasmonics 17 (2022) 1869−1888.
[53] S. Kumar, B.K. Kaushik, R. Singh, et al., LSPR-based cholesterol biosensor using a tapered optical fiber structure, Biomed. Opt. Express 10 (2019) 2150−2160.
[54] M. Puiu, C. Bala, SPR and SPR imaging: Recent trends in developing nanodevices for detection and real-time monitoring of biomolecular events, Sensors 16 (2016), 870.
[55] G. Ma, G.D. Syu, X. Shan, et al., Measuring ligand binding kinetics to membrane proteins using virion nano-oscillators, J. Am. Chem. Soc. 140 (2018) 11495−11501.
[56] S. Zhao, M. Yang, W. Zhou, et al., Kinetic and high-throughput profiling of epigenetic interactions by 3D-carbene chip-based surface plasmon resonance imaging technology, Proc. Natl. Acad. Sci. U.S.A. 114 (2017) E7245−E7254.