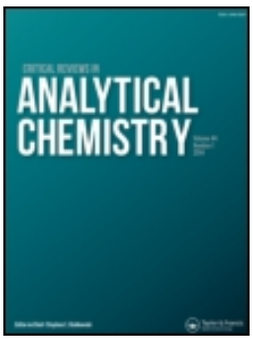

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Surface Plasmon Resonance (SPR) Biosensors in Pharmaceutical Analysis

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This review aims to highlight the applications of one of the most prominent optical biosensor technologies, surface plasmon resonance (SPR), in the drug discovery process and quality analysis of pharmaceutical compounds and their particularities. SPR assay formats and experimental issues are used for pharmacokinetic drug profiling, ADMET studies, high-throughput screening, and fragment-based drug screening, the last with an emphasis on the detection of small (drug) molecules. The classical method strengths and some applications of localized SPR and SPR imaging that are of high interest in the drug discovery process are presented, as well as possible challenges. While similar works treat separately the steps of drug discovery or focus only on the detection of drug residues in food or health safety, this review presents in a compact format the results and the progress obtained in both areas (drug discovery and quality analysis) based on the application of SPR biosensors.

Keywords: biosensors, pharmaceuticals, small molecule analysis, surface plasmon resonance (SPR)

Introduction

Since the development of the first commercial optical biosensor in the late 1980s, their applications have been described in a large number of article that cover the biomedical, pharmaceutical, and diagnostic industries, referring mainly to the interrogation and complete characterization of intermolecular interactions (Helmerhorst et al., 2012). While optical methods such as fluorescence or absorbance already have a long history, surface methods like surface plasmon resonance (SPR), surface enhanced Raman spectroscopy (SERS), and interference were developed rapidly and possess an important role in biosensing. The SPR technique is actually considered one of the most important analytical tools for interrogation of biomolecular interactions, due to its remarkable characteristics of providing highly accurate results and real-time and label-free measurements (Yadav et al., 2012). Its widespread use in research and development is perfectly justified by its characteristics, such as high sensitivity and possibility of operation within a large dynamic range and without electromagnetic interference.

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SPR phenomenon occurs when p-polarized light, under conditions of total internal reflection (TIR), strikes an electrically conducting gold layer at the interface between two media with different refractive indices, e.g., the glass of a sensor surface (high refractive index) and a buffer (low refractive index). The explanation of the SPR effect was achieved in 1968, as the energy of photons that are directly absorbed by the free electrons of a thin metallic layer (gold or silver) and converted into an evanescent field at a certain angle of incident light (Figure 1), while the first application of the SPR technique as a detection method in biosensing was mentioned only in 1983 (Wijaya et al., 2011).

Most of the available commercial SPR instruments use an optical method to measure the variations of the refraction index in the closest vicinity (~200–300 nm) of the sensor surface. Gold was chosen as the best inert metal film for surface plasmon resonance, although silver provides a better SPR effect (Liu et al., 2010; Tudos and Schasfoort, 2008). The gold surface could be modified with a self-assembling layer of long-chain thiols, to which a hydrogel (e.g., carboxylated dextran) could be further attached, providing an efficient substrate for covalent immobilization of biomolecules and a proper environment for biomolecular interactions as well.

The SPR sensorgram gives mainly two kinds of information: (a) the rate of interaction (association, dissociation, or both), which provides information on kinetic rate constants and analyte concentration, and (b) the binding level, which supplies data on the affinity constants and can be used for

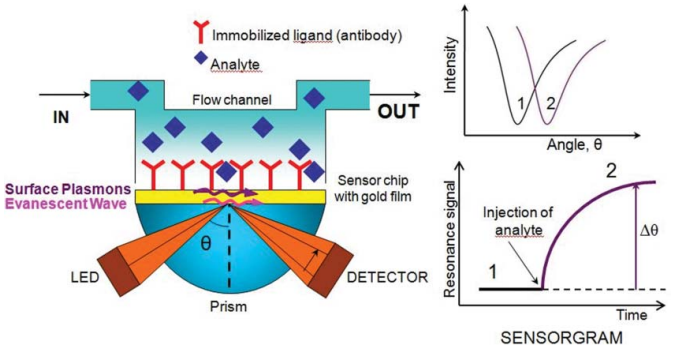

Fig. 1. Measuring principle of SPR biosensor: one binding partner (ligand) is immobilized over the sensor surface and the other partner (analyte) flows across it; the changes in the refractive index of the solution at the interface are monitored in real time using the SPR phenomenon. The sensorgram depicts changes in SPR angle in real time, with responses measured in resonance units (RU) vs. time in seconds. For SPR instruments that use light with a wavelength of 670 nm, 1 ng × mm^{-2} protein gives a signal of about 100 millidegrees (m°) angle shift. For Biacore instruments, one RU corresponds to a shift in angle of 10^{-4} degrees or to 1 pg/mm^2 of molecule on the surface (Biacore, 2003).

qualitative or semi-quantitative applications. Nevertheless, the most prominent benefit of direct detection using SPR biosensor technology is the determination of kinetics of biomolecular interactions, within which the reaction rate and equilibrium constants of interactions can be determined (Munoz et al., 2013; Wijaya et al., 2011). Kinetic constants also provide valuable data about the dynamics of the investigated biological system, which could be further applied for the selection and rational design of new molecules of therapeutic interest.

SPR biosensors, having the important benefit of completely label-free measurement, were successfully applied for a wide variety of (biological) molecules with different sizes, such as small molecules, M_r < 500 Da (Wartchow et al., 2011), to larger and more complex structures, including proteins, nucleotides, viruses, and peptides with therapeutic potential (Ananthanawat et al., 2010; Nilsson et al., 2010; Olaru et al., 2009; Suenaga et al., 2012). At the same time, the method provides comprehensive information to: (a) identify the binding typology of a pair of reactants (or more) as a function of the other interacting partner, (b) determine the affinity constants related to the interaction processes occurring during the real-time analysis, (c) quantify association and dissociation rates, and (d) determine the concentration of one/both of the interacting partners.

The application of the SPR biosensing technique as a method-of-choice for biomacromolecule characterization and/or quantification (Viel et al., 2013; Wammes et al., 2013) has required a more appropriate immobilization strategy, which limits the number of nonspecific binding events concomitant with providing a microenvironment adequate for the bioactive properties of the immobilized molecules. To overcome these issues, in particular for soluble protein immobilization, some sensor surfaces developed by GE Healthcare (formerly Biacore AB) incorporate fine layers (100–200 nm) of carboxymethylated dextran deposited onto glass-deposited gold thin films. The dextran-based sensor chips produced by GE vary the capacity by changing the percentage of dextran carboxylation. Importantly, the thickness of the dextran layer (100 nm) is perfectly compatible with the ~200 nm evanescent field and was the basis for large-scale production of these sensor chips and commercial launching of SPR instruments. The procedure of carboxy-methylation of this highly flexible polymer creates a net negative charge, which leads to the limitation of nonspecific adsorption. Moreover, it allows obtaining a sufficiently dense layer necessary for further binding of >50 pg/mm^2 of analyte, required in most of the practical applications. Similar surfaces for protein coupling were developed by Bio-Rad Laboratories, as alginate sensors chips with different lengths of the alginate layer, and by Reichert Life Sciences, which developed sensor surfaces with two-dimensional layers of carboxy-functionalized polyethylene glycol to limit nonspecific adsorption. Capture of simple lipid vesicles or previously embedded proteins is also possible through different sensors, directly onto the hydrophobic surface (available both with GE Biacore sensors and XanTec sensor chips) or by lipohilic anchors for easily capture of liposomes (GE Biacore and Bio-Rad Laboratories sensor chips).

Several SPR assay formats are available for the detection of proteins or small chemical compounds, depending on the size of the target sample (Geschwindner et al., 2012; Mitchell, 2010; Wijaya et al., 2011): direct detection (analytes with medium and large molecular weight of >10.000 Da), competition and inhibition assays (usually for low-molecular-weight analytes), and sandwich assay, for enhancing specificity and the limit of detection. More details about experimental procedures of these assays are discussed by Tudos and Schasfoort (2008).

SPR Biosensors in Pharmaceutical Analysis

Surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR) biosensors have aided the drug discovery process, mainly when applied to in vitro studies of biological and biochemical processes, and this is due to their ability to measure extremely small changes in surface refractive index (RI), binding equilibrium, and kinetics. The possibility to monitor almost any type of molecular interactions of different types of biological molecules (including serum proteins, oligomers, antibodies, and enzymes) allow (L)SPR biosensors to be used in practically every step in pharmaceutical analysis that requires kinetic profile, target identification, or characterization (Chang et al., 2013; Chen et al., 2012; Gupta et al., 2012; Hearty et al., 2012; Real-Fernandez et al., 2012; Uludag and Tothill, 2012). An increased interest in drug discovery recently focused on the impact of interaction kinetics, especially in drug-target residence time, and is based on the variation of the drug concentration over time within the body. In vitro assessment of drug-target interactions is classically quantified in terms of binding parameters such as the dissociative half-life of the drug-target binary complex (IC50) or dissociation constant (K_d). Therefore, it

might be more relevant to use in such an "open" system the interaction kinetics to analyze and optimize the equilibrium constants, dissociation constant (K_d), and association constant (K_a) (Mehand et al., 2011, 2012; Vauquelin et al., 2013).

Measuring binding kinetics using SPR is very important in establishing the duration of action and clinical benefit of a candidate drug as well the therapeutic differentiation between two or more similar drug compounds. Moreover, some slow-binding interactions could be described by a two-step mechanism that is hard to distinguish with standard assays due to the impossibility of reaching the steady state.

Without any doubt, this is the most robust and sensitive biosensing technique that has been successfully implemented in research pharmaceutical laboratories worldwide and is already a gold standard in the field. Applications of SPR include development of new biotherapeutics and drug discovery research, as well as protein activity and stability analysis in biopharmaceutical production. Identification of new biotherapeutics with emphasis on those dedicated to cancer therapy is challenging for most pharmaceutical companies nowadays (an estimation from 2008 by the International Federation of Pharmaceutical Manufacturers & Associations indicates 633 biotherapeutic medicines under development, including 254 for cancer), and this requires fast analytical characterization of biotherapeutics in early phase clinical development, which is possible with biophysical methods such as SPR.

The LSPR optical sensing method (which arises when light interacts with particles much smaller than the wavelength of the incident light) is highly localized around the nanoparticle, and this determines its higher sensitivity than SPR. Some LSPR detection methods have been developed for rapid screening of drug candidates against cytochrome P450s, which plays a key role in drug-drug interactions (Das et al., 2009). Even more sensitive, the method is also applied in biomedical diagnosis, e.g., the screening of oligomerization-blocking drugs against Alzheimer's disease or other diseases (Hong et al., 2014), more than in the drug discovery field.

Among the commercial standard SPR platforms available to date (Rich and Myszka, 2010), few comply with the demands of good manufacturing practice (GMP)/ good laboratory practice (GLP) regulations (Biacore Q, Biacore S51), favoring their direct application in pharmaceutical studies. Two directions are mainly followed and presented in the following: (a) drug screening and lead optimization, during the drug discovery complex process, and (b) quality analysis of drug residues in different matrices, as a result of drug treatment or abuse, and summarized as well in Table 1.

Drug Discovery

Pharmacokinetic Drug Profiling

One of the most important aspects of pharmacokinetic research (in particular for drug compounds administrated by the oral route) is related to drug transport from the site of administration into the systemic circulation, since a drug is considered absorbed only when its entrance into the blood capillaries is completed. At the same time, drug absorption also depends on a number of physico-chemical factors, among which the lipophilicity and solubility are the most important. Gathering information about the membrane structure and its functionality is important, since these affect the transport across membranes and in some cases are the action spot for the therapeutic effect (Alqahtani et al., 2013; Roberts and Hall, 2013; Siissalo and Heikkinen, 2013).

Within the group of predictive in vitro methods (Alqahtani et al., 2013) used to assess drug permeability (including here the "classical" known methods such as octanol/water distribution, cyclohexane/water distribution, immobilized artificial membranes (IAM), immobilized liposome chromatography (ILC), micellar electrokinetic chromatography (MEKC), and biopartitioning micellar chromatography (BMC)), SPR biosensors have been more recently considered (Danielson, 2009; Guo, 2012).

As such, drug permeation into biomembranes has often been characterized using biomimetic (SPR) sensors with immobilized liposomes or cell membrane fragments directly binding onto the sensor surface. A large panel of drugs with high transcellular absorption could be identified on the basis of the binding assay, even though the binding kinetic is not always related to the transcellular transport.

Liposomes have been directly attached to the SPR biosensor surface, and the interactions between several drugs and the immobilized liposomes have been monitored in real time (Baird et al., 2002; Danelian et al., 2000) and compared to the fraction absorbed in humans (% *Fa*). A similar SPR study was completed on a larger panel of diverse drugs varying in molecular weight (from 138.1 to 664.8 Da) and chemical functionality, totaling 85 compounds. The aim of this study was to establish a classification of drug compounds according to their apparent liposome-binding affinity and to compare dissociation phase kinetic data with lipid retention measurements obtained from a parallel artificial membrane permeability assay (PAMPA) (Abdiche and Myszka, 2004). The majority of drugs with high transcellular absorption could be identified on basis of the binding assay; even though the binding observed by the sensor may not always be related to transcellular transport. The partition coefficients measured in the liposome system may not always reflect biological permeation but rather drug interactions with the membrane surface (Malkia et al., 2004). Nevertheless, information of such interactions could have implications for drug-induced membrane effects.

Immobilization of liposomes on the SPR sensor substrate is a rapid and reproducible process, and the obtained detection platform could be used for studies of interactions with various pharmaceutical compounds. Most of the curent investigations and biomolecular analysis as well kinetic studies in drug development are completed using liposome-functionalized SPR sensors.

Table 1. Applications of SPR biosensors in pharmaceutical analysis

SPR biosensors in pharmaceutical analysis		Aim	Investigated pharmaceutical compounds	References
Drug discovery	Pharmaco-kinetic drug profiling	· Predictive in vitro drug permeability · Passive transport of drugs through membrane · Characterization of membrane binding affinity	– Salmeterol, propranolol, alprenolol, oxyprenolol, metoprolol, verapamil, pindolol, despiramine, dibucaine, imipramine, naproxen, suprofen, tetracaine, tolemtin, ketoprofen, homochlorocyclizine, hydrochlorothiazide – Sulfasalazine, atenolol, creatinine, D-glucose, urea, mannitol – Diverse drugs with different *Mw* (from 138.1 to 664.8 Da) and chemical functionalities – Drugs with high transcellular absorption.	Abdiche and Myszka, 2004; Baird et al., 2002; Danelian et al., 2000; Frostell-Karlsson et al., 2005; Kim et al., 2004
	High-throughput screening (HTS)	· Target and assay validation	– Candidate drugs and drug fragments – Primary screening of large libraries comprising small (drug) molecules	Giannetti, 2011; Mayr and Bojanic, 2009
	Fragment-based drug design (FBDD)	· Identification of lead structures · Affinity ranking and kinetics for small molecules binding to target protein	– Small molecules (*Mw* 100–300 Da) – Active compounds with *Mw* < 500 Da, LogD < 5, solubility > 10 μM – Thrombin inhibitors – HIV protease inhibitors – DNA-binding drugs and novel inhibitors of the bacterial enzyme DNA gyrase	Anraku et al., 2010; Bai et al., 2013; Boehm et al., 2000; de Kloe et al., 2009; Gambari et al., 2000; Geschwindner et al., 2012; Lee et al., 2013; Lin et al., 2011; Mani et al., 2011; Markgren et al., 2002; Vaisocherova et al., 2009
	Early ADME/T studies	- Rapid in vitro assays for defined ADME/$T_{parameters}$, as protein-binding studies, for potential drug candidates - Prediction/measuring of drug/human serum albumin binding.	– Small molecules (130–800 Da) – Drugs binding to alpha(1)-acid glycoprotein (AGP)	Frostell-Karlsson et al., 2000; Rich et al., 2001
Quality analysis	Quality control	· Label-free and rapid detection of drug residues in food or clinical samples	– Antibiotics (sulfonamides, sulfadiazine, sulfamethazine, β agonists, chloramphenicol, streptomycin, benzimidazolecarbamate) – Hormone-like substances – Vitamins – Pharmaceutical proteins (CTLA-4 fusion protein)	Keegan et al., 2009; Narsaiah et al., 2012; Petz, 2009; Rebe Raz et al., 2009; Vyas and O'Kane, 2011; Vyas et al., 2012; Wang et al., 2009; Yuan et al., 2009

In summary, several types of information are gained during the SPR interaction studies between biomimetic membranes and drugs, such as:

i. Binding kinetic of the drug product to the phospholipidic substrate and its affinity for the biomimetic membrane (Lombardi et al., 2009).
ii. Drug partition within the membrane: determination of its lipophilicity in terms of partition coefficients "log P" and characteristics of drug distribution in lipid (oil) and aqueous phase. Thus, the lipophilic feature could be determined for some of the already existing drugs as well for putative drugs.
iii. Differentiation and prediction of the (drug) fractions absorbed (% *Fa*) in the intestines after the oral administration (Frostell-Karlsson et al., 2005).
iv. Selection of potent pharmaceutical compounds during the drug development process and establishment of the relation between the molecular structure and action dose, based on the SPR kinetic profiling (association and dissociation constants).

In conclusion, the quantitative measurement of passive transport is readily predicted using the liposome immobilized SPR system, even though this configuration showed different interactions with orally absorbed drugs according to physicochemical properties of lipids, such as lipid structure, lipid ordering, and lipid fluidity. Oral drug permeability from the small intestine was estimated based on the interaction between drugs and brush border membrane (BBM) surfaces immobilized on an SPR biosensor chip (Kim et al., 2004). BBM vesicles isolated from Sprague-Dawley (SD) rats were immobilized on an SPR sensor surface and the binding responses were correlated with the fraction absorbed in humans for transcellularly absorbed drugs. Similar to the SPR liposome studies mentioned above, the active and paracellular-mediated transport mechanisms are difficult to investigate for this version as well, even though the biological environment created seems to be more favorable, at least in the beginning.

High-Throughput Screening (HTS)

High-throughput screening (HTS) is a well-established process for lead discovery in the pharmaceutical industry, comprising the screening of large chemical libraries for activity against biological targets with the use of automation, miniaturized assays, and large-scale data analysis (Mayr and Bojanic, 2009). HTS is defined by the large number of compounds tested, in the range of 10,000–100,000 per day, useful for discovering ligands for receptors, enzymes, ion-channels, and other pharmacological targets and pharmacologically profiling cellular and biochemical pathways of interest. Typically, HTS assays are performed in microtiter plates with different well formats and variable volumes per well: 96 wells (100–200 μL), 384 wells (30–100 μL), and 1536 wells (2.5–10 μL). Analytical systems for drug screening should allow high throughput with a broad variety of compounds of different origins, e.g., combinatorial libraries, new synthesized forms, and natural sources (Danielson, 2009; Wilde and Link, 2013).

Biophysical methods such as X-ray, nuclear magnetic resonance (NMR), and SPR have generated a promising alternative to HTS in cases where conventional HTS for biochemical targets has not delivered good compounds (hits) as starting materials for lead optimization (Mayr and Bojanic, 2009). Thus, the trend of key pharmaceutical companies was to focus on the screening of drugs and drug fragments instead of the ultrahigh-throughput screening (uHTS), determining a new, favorable position for biosensors as high-information-content screening tools, able to detect a large variety of drug candidates. For example, SPR biosensing technology is in widespread use due to its fast selection of fragments with binding affinity. It provides kinetic data on biomolecular interactions, allowing researchers to quantify the binding characteristics of lead compounds with their targets in terms of affinity, specificity, and association/dissociation rates in parallel. Quantitative kinetics of compound binding can be used to better understand the binding mechanisms and processes, as well the effect induced by the modification of chemical structure. Association and dissociation rates can be varied independently for a specific lead series, resulting in the rapid evolution of sub-nanomolar affinity leads (Giannetti, 2011).

Fragment-Based Drug Design (FBDD)

Fragment-based drug design has gained much attention since the theoretical concept was initially suggested in 1981. Fragments are molecules with a molecular weight of 100 to 300 Daltons, simpler (fewer functionalities than larger molecules), and more soluble than drug-like compounds. Biosensor-based fragment screening is an established practice in the drug discovery process (Wartchow et al., 2011), numerous studies indicating its successful application for identification of lead structures in medicinal chemistry (de Kloe et al., 2009; Geschwindner et al., 2013). In contrast to conventional HTS, where typically 10^5–10^6 drug-like compounds are screened to find relatively potent leads with K_d values ideally less than 1 μM (Proll et al., 2009), in the case of FBDD a lower number of small chemical structures are screened and the low-affinity binders are identified, which afterwards can be combined to gain a high-affinity drug lead. Practically, this technique measures the direct interaction of a (drug) fragment with a target protein immobilized on a biosensor, and further interpretation of the kinetic constants is based on the binding data (Christopher et al., 2013). Detection and analysis of molecular interactions allow the screening of fragments otherwise undetected by HTS methods.

Due to the major importance of the analysis of small-molecule interactions in drug discovery and development (including fragment screening), a dedicated SPR instrument was developed by Biacore AB company (actually GE Healthcare). The SPR platform, Biacore S51, includes increased sensitivity, larger sample handling capabilities, and automated data processing to improve throughput. Dedicated to small molecule detection, the instrument was optimized to improve the signal-to-noise ratio and sample delivery and is capable

of monitoring the binding of molecules with Mw <90 Daltons directly to macromolecular targets.

Direct monitoring of low-molecular-weight molecules is sometimes difficult. To overcome this issue, different experimental procedures have been used, e.g., immobilization methods based on a dextran layer to increase the binding surface capacity or increasing the number of binding sites by repeating the sequence of interest several times in each probe (Sipova and Homola, 2013). Furthermore, small drug molecules have been detected directly by SPR, such as the thrombin inhibitor (Bai et al., 2013; Lin et al., 2011; Mani et al., 2011) that prevents the blood coagulation in different medical treatments. Direct binding assay and competitive assay formats (based on the sample mixture with hirudin) have been used for the study and found suitable for screening, since the tested substances with known affinity to thrombin have been detected within the 1–10 μM concentrations range. Similarly, HIV protease inhibitors, one of the most promising drugs for AIDS treatment, were screened with the SPR technique (Anraku et al., 2010; Lee et al., 2013; Vaisocherova et al., 2009). The biosensor-based direct binding assay provides high-resolution information on the interaction kinetics and specificity of the interaction between a large number of structurally diverse human immunodeficiency virus type 1 (HIV-1) protease inhibitors and an immobilized enzyme (Markgren et al., 2002).

Novel inhibitors of the bacterial enzyme DNA gyrase (Boehm et al., 2000) as well the characterization of DNA-binding drugs (Gambari et al., 2000) were screened with SPR biosensors. Interactions between DNA biotinylated probes immobilized on an SPR sensor surface and the model were directly monitored, allowing rapid analysis of molecular interactions. Chemically modified nucleic acids have been investigated targeting the identification of potential targets for the antisense drugs (Ananthanawat et al., 2010). More applications of SPR interactions of nucleic acids and various compounds of medical interest are indicated in the review of Sipova and Homola (2013).

SPR imaging technology for high-throughput screening of large collections of small molecules was more recently considered. The method provides the same type of quantitative data as obtained in biosensing with SPR spectroscopy but possesses a higher spatial resolution for monitoring adsorption (down to 4 μm over a large area of a sensing surface). It allows the simultaneous measurement of thousands of biomolecular interactions, e.g., the detection of up to 9216 target-ligand interactions on a single array could be completed within a few hours, and it is therefore suited to library sizes of up to 10^5 (Neumann et al., 2007). A few recent examples include the screening of the interaction of several small molecules, referred to as G4-ligands, in G-quadruplex DNA (Pillet et al., 2011) and estrone detection (Karabchevsky et al., 2013).

ADME/T Profiling

Without neglecting the "weight" of the data generated by the HTS, an important aspect of the drug discovery process is the critical evaluation of the results, with an emphasis on the interaction specificity and predictive adsorption, distribution, metabolism, excretion, and toxicity (ADME/T) profile, which determines the bioavailability and effectiveness of the drug compound. Biacore technology is a feasible tool for cost-effective in vitro characterization of a potential drug candidate in early ADME studies, through serum protein and lipid-binding assays as well as in animal model systems. Comprehensive SPR biosensor analysis was performed on the interaction between immobilized human serum albumin and drug compounds (about 100 different representatives; Frostell-Karlsson et al., 2000) and to a lesser extent for prediction of human serum albumin (HSA) binding levels (Chang et al., 2009; Day and Myszka, 2003; Tomassetti et al., 2013; Zhou et al., 2008). The affinity constants determined from the biosensor assay corresponded with affinities determined by other methods. Using warfarin as a test system, the SPR Biacore technology was validated for efficient determination of binding constants for drug-HSA interactions, concomitant with the ability to directly examine the binding of small molecules (130–800 Da) (Rich et al., 2001). Limitations of traditional methods such as equilibrium dialysis (ED) and ultrafiltration (UF) in establishing the plasma-protein binding (PPB) parameter, compared with modern techniques as well with SPR biosensor technology, were recently presented by Vuignier et al. (2013).

Toxicity is responsible for many compounds failing to reach the market and for the withdrawal of a significant number of compounds from the market once they have been approved. It has been estimated that ~20–40% of drug failures in investigational drug development can be attributed to toxicity concerns (Guengerich, 2011). Toxicological assays useful in early discovery process are hERG (or gene KCNH2) and other cardiac ion channels**,** genetic toxicology, and target organ/cytotoxicity addressing issues like cardiotoxicity, teratogenicity, mutagenicity, hepatotoxicity, neurotoxicity, and nephrotoxicity, and these are regularly completed with biochemical toxicity tests such as cellular adenosine triphosphate content (ATP assay) and mitochondrial respiration assay (MTT assay). There are a limited number of articles that report studies of drug toxicity profiling using SPR biosensors (Nishijima et al., 2010; Umezawa et al., 2002) and cytotoxicity studies using SPR cell-based assays (Michaelis et al., 2013), while most of the articles mention other types of biosensors (Antczak et al., 2012; Wang et al., 2013).

Quality Analysis

Screening of new drugs is not comparable to routine quality analysis performed with biosensors for control/quantification purposes, since in most cases it is targeting the detection of a single compound in the same matrix (Wang et al., 2009). The SPR biosensing technique is extensively used in quality control laboratories worldwide, targeting label-free and rapid detection of drug residues in food and clinical samples. SPR commercial bioassays were successfully used for detection of synthetic hormone-like substances and antibiotics (sulfonamides, sulfadiazine, sulfamethazine, β agonists, chloramphenicol, streptomycin) in meat and honey (Narsaiah et al., 2012; Rebe Raz et al., 2009), as well for

vitamin quantification in fortified foods (Petz, 2009; Vyas and O'Kane, 2011; Vyas et al., 2012). Among the ~25 producers of SPR instruments (McWhirter and Wahlstrom, 2008), Biacore Q and Q flex kits occupy a distinct position in food analysis due to their cumulative advantages such AOAC Performance Tested Methods, minimal sample preparation, and reduced analysis time compared with traditional microbiological assays. Also, some improvements of SPR assays in the preparation procedure and sensor surface regeneration have been reported for rapid antibiotics screening (benzimidazole carbamate and cloramphenicol) in food samples (Keegan et al., 2009; Yuan et al., 2009). Wang et al. (2009) reported the development of biosensor-based SPR technology for biological quantification and quality control of pharmaceutical proteins. Fast identification of drug residues in clinical samples (human saliva or urine) was also successfully tested with SPR immunosensors, targeting low levels of steroids or amphetamines (Lee et al., 2013; Mitchell, 2010).

Conclusions and Future Trends

The net advantage of the SPR biosensing method versus other optical (bio) sensors is that the entire interaction between the analyte and the ligand is monitored in real time, without any molecule labeling; moreover, reduced analysis time and simple procedures for sample preparation are added advantages when compared with traditional analytical methods.

Within the drug discovery domain, SPR-based screening methods seems to be the most promising tools for high throughput of novel pharmaceutical forms, since the interactions of low-molecular-weight compounds could be monitored even at low concentrations (especially in a direct-binding assay format, if the ligand is a high-molecular-weight compound). The detection limit is highly dependent on the sensor type and its surface properties, affinity to the immobilized binding partner, and molecular weight of the ligand. On the other hand, the main disadvantage of SPR in HTS is that the intrinsic activity of the binding partner can not be directly characterized since it requires more complex experimental procedures to differentiate between the agonists and antagonists. Low-molecular-weight interaction partners and identification of unknown substances could be directly determined by the combination of SPR with matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) (Borch and Roepstorff, 2010; Kim et al., 2012). New trends of SPR instrumentation as well novel surface functionalization strategies of SPR sensing surfaces are reviewed by Abbas et al. (2011) and Wijaya et al. (2011) respectively. For instance, SPR devices have risen to the demand of array-based sensing with the introduction of SPR imaging, which permits multiple assay spots to be monitored simultaneously (Turner, 2013). Coupling of SPR with other techniques, emerging technologies, and novel sensing materials will no doubt be continued, contributing to the development of sustainable in vitro methods and improved drug candidates. A robust SPR method for the fast evaluation of a candidate drug toxicological profile could be also developed.

As SPR biosensor technology already occupies a distinct place in pharmaceutical analysis, the most challenging future issues are improvements of already established methods for assay formats, limits of detection, and sensor surface regeneration. Under this continuous development, standardization of SPR pharmaceutical methods could be the next aim, contributing to the global harmonization of regulations in the food and public safety domains.

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