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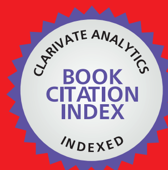

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Chapter 9

Ion-Pair Spectrophotometry in Pharmaceutical and Biomedical Analysis: Challenges and Perspectives

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Additional information is available at the end of the chapter

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Abstract

Experimental and theoretical studies of the mechanisms that underlay ion-pair formation, their properties and applications in various fields have been and still are focused by researchers since the introduction of the concept in 1926, by Bjerrum. Ion pairs are distinct chemical entities, electrically neutral, formed between ions of opposite charge and held together by Coulomb forces, without formation of a covalent bond. Investigation methods used are various, from classical conductometric measurements to up-to-date methods, such as spectrophotometry, chromatography and capillary electrophoresis. In the pharmaceutical field, ion pairs were used to develop methods of separation, identification and assay for the active substances in complex matrices, to obtain pharmaceutical formulations with controlled release and to explain the mechanisms of transport and action for certain drugs. The chapter is an attempt to describe new trends in the spectrophotometry of ion pairs and their applications in the pharmaceutical field. The development of the concept and types of ion pairs are first presented; further, examples of applications using molecular absorption, fluorimetry and resonance light scattering spectrophotometry are presented. Based on the literature data and the authors' experience in the field, challenges and perspectives in the ion-pair spectrophotometry are also considered.

Keywords: ion-pair spectrophotometry, pharmaceuticals, UV-Vis absorption, fluorescence, resonance light scattering, resonance Rayleigh scattering

1. Introduction

Ion-pair spectrophotometry refers to analysis methods based on the optical properties of the ion pairs. Infrared, nuclear magnetic resonance and Raman spectrometry are the methods generally used to investigate the structure of the ion pairs and molecular and atomic absorption, fluorimetry and resonance light scattering are used as assay methods.

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Known also as *ionic associations* or *ionic association complexes*, ion pairs are pairs of oppositely charged ions held together by Coulomb attraction without formation of a covalent bond [1]. The lifetime of an ion pair was determined to be of at least 10^{-5} seconds, equivalent to about 10^{8} molecular vibrations, demonstrating that ion pairs can be considered as independent species [2].

The inclusion of a substance in an ionic association causes changes in its physical-chemical properties without changing its structure, because an ion pair is electrically neutral and has increased lipophilicity compared with the free ions in its composition [3]. The optimum experimental conditions for a quantitative ion-pair equilibrium (solvent, pH, ionic strength) are easily settled. The selectivity of the methods can be increased by selecting the optimum reagent (counterion, ion-pair forming reagent) and the subsequent extraction of the ion pair in an organic phase.

The significant number of the scientific papers published indicates the appropriateness of ion pairing in solving important issues in the pharmaceutical field, especially in analytical chemistry, biochemistry and pharmaceutical technology. Ion pairs are used for the development of new pharmaceutical forms with controlled release, especially for peptides [3–5]. In this case, one of the main advantages is the unmodified pharmaco-toxicological profile of the active substance after ion pairing, because it does not suffer structural changes. The stability [6] and bioavailability [7, 8] of the drugs can be improved. A series of kinetic studies proposed the ion-pair formation as an absorption mechanism for the pharmaceutical substances [9, 10]. Investigations on DNA stability in various matrices [11], protein determination [12] and synthesis of ion-pair receptors based on biological models [13] are important applications of ion-pair equilibrium in biochemistry. Ion-pair–based assay methods are proficient in both isolation, identification and quantification of certain substances of biomedical interest [14]. Over time, titrimetric [15, 16], gravimetric [17, 18], electrometric [19, 20], spectrophotometric [21, 22] and chromatographic [23, 24] methods based on the ion-pair formation were developed.

Classical and modern as well, ion-pair–based spectrophotometric methods had a dynamic evolution over the time. On the one hand, this is due to the elucidation of the mechanisms underlying the formation of ion pairs, and thus, the setting of the experimental conditions, which allow the obtaining of ion pairs for all types of substances, is easier; in this regard, computational chemistry is a very useful tool. On the other hand, the synthesis of new pharmacologically active molecules at very low concentrations requires sensitive analysis methods. Among them, less used spectrophotometric techniques, such as resonance light scattering, have found interesting applications when ion pairing was taken into account.

2. Fundamentals of ion pair

2.1. History of the ion-pair concept

The ion-pair equilibrium has been first considered for inorganic ions, being an important step in the study of the electrolyte solutions. The history of the ion-pair concept starts in 1887 with Arrhenius, who structured the theory of electrolytic dissociation. Debye and Hückel

defined in 1923 the activity coefficients and deduced the homonym equation that allows the assessment of those coefficients in aqueous solutions of electrolytes [25]. In 1926, Bjerrum introduced an association constant in the Debye-Hückel equation and demonstrated that ion-pair equilibrium is dependent on the dielectric constant of the solvent, on the temperature and on the size of the ions. For his theory, he considered spherical, nonpolarizable interacting ions [26]. Thus, the existence of ion pairs was accepted in low dielectric constant solvents. Subsequently, studies were performed in order to elucidate the existence of solvent molecules in the ion-pair structure [27]. Most of the experimental data used to confirm theoretical studies on ion pairing were conductivity measurements.

The subsequent development of organic synthesis and the physical-chemical study of association of more complex molecules, concomitant with the development of new analysis methods (spectrophotometry, chromatography), indicated that, when forming an ion pair, the interacting ions cannot be considered as rigid and spherical [28]. In 1967, Higuchi et al., considering the volume and charge distribution over the ions, studied how a contact ion pair can be solvated in various solvents. For the ion pairs formed between a large lipophilic cation and a small anion, the high negative charge per unit area, lead to the solvation with electrophilic molecules, such as chloroform, phenols and alcohols. The high negative charge on the surface of the ion pairs formed between a small cation and a large lipophilic anion induce the solvation with nucleophilic molecules, such as ethers, ketones, amides and phosphate esters. For the ion pairs formed between two large ions, no significant solvation was observed [29].

Hydrophobic interaction, typical for large unhydrated (hydrophobic) univalent ions, was proposed as a mechanism of ion-pair formation in aqueous solution by Diamond [30]. The driving force for the ion pairing is the water molecule preference to interact with itself by hydrogen bonding. The equilibrium is named *water structure-enforced ion pairing* and the complexes formed accordingly—*water structure-enforced ion pairs*. Thus, starting from this point, the existence of the ion pairs in water became an accepted fact.

When the interaction between the oppositely charged ions is strictly electrostatic, no new electronic bands appear in the absorption spectrum [28]. Spectral changes reported in the studies indicate that, for the ion pairs formed by organic ions, additional interactions (aromatic stacking, charge transfer, hydrogen bonding) might exist.

Aromatic stacking is indicated by a hypochromic effect in the absorption spectra and was demonstrated by thermodynamic studies for the interaction between organic species containing aromatic structures [31]. Considered to be a result of a non-classical hydrophobic effect, the *stacking* of the *aromatic* rings is determined by the interaction between the partial charges (positive and negative) that exists on the atoms situated in adjacent aromatic rings.

The redistribution of the charge between the ions (charge transfer) is identified spectrophotometrically by a hypsochromic (blue shift) or bathochromic (red shift) effect in the UV-Vis region, depending on the medium polarity. This type of interaction can be predicted by theoretical calculations, based on charge density and molecular orbital theory [32, 33]. The ionic associations based on such interaction have been named *ion-pair charge-transfer complexes* [32].

Similarly, it was proved that ion pairs can be formed also by the interaction between an acid and a base by *proton transfer* [34].

Thus, nowadays, it is generally accepted that electrostatic interactions, hydrophobic interactions and proton transfer are the main mechanisms involved in the ion-pair formation and that the ion pair stability depend on the structure and size of the ions, on their acid-base and hydrophobic properties and on the solvent nature as well.

The methods currently used for the study of the ion-pair equilibrium are spectrophotometry (molecular absorption, resonance light scattering and fluorescence), conductometry [35], chromatography [23, 24, 36] and capillary electrophoresis [37].

The development of computational chemistry makes possible simulation of associations between complex molecules. Thus, in silico investigations became a valuable tool in the study of the ionic association equilibrium. Such studies can explain the formation of a certain complex, or predict it, and are commonly validated by spectrophotometric methods.

2.2. Types of ion pairs

The formation mechanism and the structure of the ion pairs were established concurrently with the elucidation of the interaction types of the ions in solution. Considering the solvation, ion pairs exist in the *tight* (*contact, intimate*) form (no solvent molecule is involved in the ion pair) and in the *loose* form (one or more solvent molecules are included in the ion pair). Depending on the number of the solvent molecules involved, ion pairs are of *solvent-sharing* type (a single solvent molecule is included) and *solvent-separated* ion pairs (when more than one solvent molecule is involved) [1].

Considering the structure of the ions involved in the ion-pair equilibrium, an overview of the literature published allowed the identification of three categories: (a) inorganic ion pairs (both ions are inorganic), (b) ion pairs formed between an organic molecule in ionized form and an inorganic ion and (c) organic ion pairs (both ions are organic substances in ionized form). The inorganic ions can be included in an ionic association in the free form or as inorganic complex. The organic substances are transformed in the ionic form based on their acid-base properties, by selecting the optimum pH, or after a complexation reaction with an inorganic ion. The inorganic ion pairs are intensively studied by physical chemistry, for the theoretical background of the mechanisms of ion pairing. The ion pairs that contain an organic ion are more used in the pharmaceutical field.

The solubility of the ion pairs in the selected solvent can be also a criterion to classify the ion pairs. Thus, two categories can be discerned: insoluble and soluble ion pairs. Insoluble ion pairs are used in the assay of the pharmaceuticals by gravimetric methods [17, 18, 38] and atomic absorption spectrometry [39, 40] and also in pharmaceutical technology for drug release systems [5]. The applications that rely on the formation of soluble ion pairs are most numerous.

As an ion pair is electrically neutral, the number of ions involved depends on their charge. Frequently encountered in literature are binary ion pairs, formed between ions with the same charge, and ternary ion pairs, which contain one divalent ion and two monovalent counterions.

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3. Spectrophotometric applications of ion pairing in pharmaceutical analysis

3.1. Molecular absorption spectrophotometry

Most of the ion-pair spectrophotometric assay methods published in pharmaceutical field are based on UV-Vis molecular absorption. These methods are robust, easy to perform, sensitive, accurate and precise. In association with an organic dye, pharmaceutical substances with no characteristic visible spectrum can be detected in this region.

Widely used are the extractive spectrophotometric methods: the ion pairs are extracted in an organic solvent, and the extract is further analyzed. For the quantitative extraction in the selected organic solvent, the optimum pH value, concentration of the reagents and ionic strength must be established.

The selection of the counterion should consider that bulky, univalent and having the charge distributed over the whole ion reagent has the best capacity to form ion pairs. With respect to the geometry of the counterion, planar types of organic dyes are appropriate for developing ion-pair absorption spectrophotometric methods [41]. Computational chemistry is a useful tool to evaluate the volume, geometry and charge density of the studied substances. By correlating these data with the results of the studies on the solvation of different types of ion pairs [29], the selection of the optimum solvent for the extraction is simplified.

The formation of the ion pair can be revealed spectrophotometrically by a shift of the absorption peak of the chromophore. As an example, the spectral changes that appeared at the formation of terbinafine-methyl orange (TBF-MO) ion pair in chloroform were used for the assay of terbinafine by ion-pair absorption spectrophotometry by Florea et al. [42]. MO is a planar dye containing aromatic rings, and the formation of TBF-MO ion pair is accompanied by a blue shift (from 502 to 408 nm) and hypochromic effect for the visible peak of MO (**Figure 1**). These spectral changes indicate the stabilization of the ion pair by aromatic stacking [31].

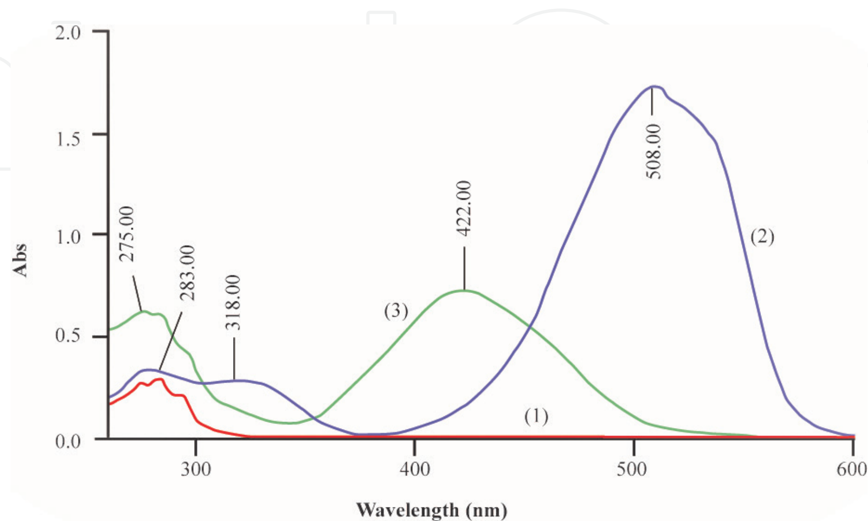

Figure 1. Molecular absorption spectra of TBF (1), MO (2) and TBF-MO ionic association (3) (from Ref. [42], with permission).

Bromocresol purple (BCP) [43] and alizarin red [44] were also used as counterions in the assay of TBF using extraction methods.

As counterions, the chain-type reagents having long alkyl groups are also useful. They are bulky and univalent, but their charge is not distributed over the whole ion. Even so, the main limitation arises mostly from the fact that they are colourless; therefore, they can be used as ion-pair reagents for the assay of coloured substances.

Hexadecyltrimethylammonium bromide (CTAB) was used to develop an extractive spectrophotometric method for the assay of nimesulide (NS) by Florea et al. [45]. As CTAB is a chain-type reagent, with no aromatic rings in the structure and no characteristic spectrum in UV-Vis region, NS in its ionized form is the reagent having a peak in visible range. Therefore, when the ion pair is formed, a red shift and hyperchromic effect appeared (**Figure 2**).

Sulfonephthalein dyes, such as bromocresol green (BCG), bromocresol purple (BCP) and brilliant blue G [46], were also used as counterions in the assay of NS using extraction-free methods. A comparison of the experimental data indicated a larger linearity range for the method based on the ion pair formed with CTAB.

Because extraction is a laborious procedure, the trends are to develop non-extractive (extraction-free) ion-pair–based spectrophotometric methods in nonaqueous or aqueous solutions. Generally, by dissolving the substances in the organic solvents, the ion pairs are formed mainly by a proton transfer mechanism.

Limitations in the development of non-extractive methods arise mainly from the physical-chemical properties of the reagents, namely, their solubility in the appropriate solvents.

Literature data generally resulted in narrower linearity ranges for the non-extractive methods compared with the extractive ones. Some examples are presented in **Table 1**.

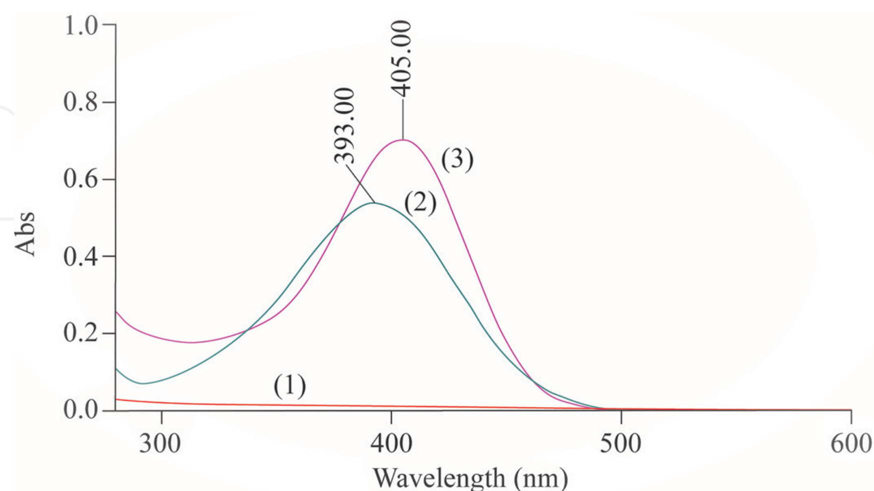

Figure 2. Molecular absorption spectra of CTAB (1), NS (2) and CTAB-NS (3) (from Ref. [45], with permission).

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Analyte	Method	Counterion	Solvent	Linearity range (μg/mL)	Reference
Desloratadine	NE	Eosine	H_2O	0.31–2.81	[47]
	E	$[Co(SCN)_4]^{2-}$	$CHCl_3$	0.5–3	[48]
Losartan	NE	Eosine	H_2O	2.5–20	[47]
	E	Calmagite	$CHCl_3$	10–100	[49]
		Orange II	$CHCl_3$	10–100	[49]
Levofloxacin	NE	BCG	CH_2Cl_2	1–20	[50]
	E	Bromophenol blue	$CHCl_3$	1.85–31.5	[51]
		BCG	$CHCl_3$	1.85–25	[51]
Ampicillin	NE	Pyrocatechol violet	H_2O	0.2–28	[52]
	E	$[Mo(SCN)_6]^-$	CH_2Cl_2	1.5–77.5	[53]
Amoxicillin	NE	BCG	$(CH_3)_2SO$	1–13	[54]
	E	Methylene blue	$CHCl_3$	3.5–90	[55]

Table 1. Examples of extractive (E) and non-extractive (NE) ion-pair spectrophotometric methods for the assay of pharmaceutical substances.

3.2. Fluorescence spectroscopy

Among spectrophotometric methods, fluorimetry distinguishes itself by high sensitivity and specificity. In pharmaceutical sciences, fluorescence spectroscopy is an irreplaceable tool in the study of biochemical processes occurring at the cellular level. Substances having intrinsic fluorescence, named *fluorophores*, have characteristic structural features (rigid, plane structure with conjugated double bounds) and exhibit specific excitation (absorption) and emission (fluorescence) wavelengths, thus explaining the high specificity of the method [56]. Various interactions of the fluorophore with the surroundings can lead to a decrease of the fluorescence intensity. This effect is called quenching and can be used for quantification purposes, primarily for the determination of anions [57]. Molecular mechanisms such as the interaction with electron-deficient molecules (quenchers) in the excited state of the fluorophore (collisional quenching) or in the ground state (formation of non-fluorescent complexes with quenchers), together with different non-molecular effects, can be involved in the quenching process [56].

Ion-pair fluorescence assay methods are generally based on quenching. In the ion-pair structure, if one of the ions is a fluorophore, the counterion can act as a quencher. For a certain concentration range, the decrease of the fluorescence intensity is proportional with the analyte concentration. The development of these methods takes into consideration the same experimental conditions presented at Section 3.1, to obtain a quantitative ion-pair equilibrium (pH, ionic strength, solvent), but it is conditioned by the selection of an optimum fluorophore. Organic substances with native fluorescence that can be used as counterions are few; therefore, there are not many published applications. Literature data on ion-pair fluorescence methods for the assay of pharmaceutical substances are summarized in **Table 2**.

Fluorescent reagent	Analyte	Reference
Extractive methods		
Erythrosine B	Erythromycin	[58]
9,10-Dimethoxyanthracene-2-sulphonate	Imipramine	[59]
	Desipramine	
	Amitriptyline	
	Nortriptyline	
	Clomipramine	
	Doxepin	
Nonextractive methods		
Eosine	Astemizole, terfenadine, flunarizine as chelates with Pb^{2+}	[60]
	Amitriptyline	
	Clomipramine	[61]
	Rosiglitazone	[62]
	Pioglitazone	[63]
	Albendazole	[64]
Eosine (as chelate with Pd^{2+})	Ciprofloxacin	[65]
	Norfloxacin	
Safranin T	Meloxicam	[66]
4,5-Dibromofluorescein	Ceftazidime	[67]
	Ceftriaxone	
	Cefoperazone	

Table 2. Examples of extractive and non-extractive ion-pair fluorescence methods for the assay of pharmaceutical substances.

Berberine, an isoquinoline alkaloid, is a pharmacologically active fluorophore, with potential therapeutic effect in various diseases (Alzheimer's disease, cancer, viral infections, etc.). In order to get deeper insights into the details of its biological activity, the effect of the ion pairing on its fluorescence properties was studied using chloride and perchlorates anions [68]. Nanoparticles containing berberine-tetraphenylborate ion pair were prepared, and their ability to cross the cell membrane of cancer cells was studied by Soulié et al. [69].

Lately, the research in nanoscience opened an even wider pathway in fluorescence studies involving ion pairing. Quantum dots (QDs) are prone to exchange electrons with their complementary partners (acceptors or donors) upon excitation that can be transduced into detectable fluorescent signals [70]. Thus, sensitive assay methods can be developed by using QDs capped with different ligands in ionized form. An example is the determination method developed for albendazole, using glutathione-capped cadmium telluride QDs (GSH-CdTe QDs).

Ion-pair equilibrium takes place between albendazole in cationic form and anionic sites at the QD surface, and the effect was a decrease of the fluorescence intensity of capped CdTe QDs [70].

Various applications based on ion-pair equilibrium with fluorescence properties were developed along the time for the characterization of biomolecules in complex biological matrices by flow cytometry [71] and also for kinetic studies using fluorescence microscopy [72].

3.3. Light scattering spectrometry

Light scattering was observed by the Irish physicist John Tyndall in the late 1860s. The eminent British physicist Lord Rayleigh (John Strutt) developed the theoretical basis of electromagnetic wave interaction with particles smaller than the wavelength in the following decades (1870–1899). Now, the scattering of light by particles in a suspension is accepted to be elastic (without change in the wavelength of the incident light) and inelastic (the incident wavelength and the scattered one are different). Rayleigh scattering theory was developed for wavelengths much higher than the dimensions of the scattering particles.

Light scattering is widely used since the 1950s in chemical analysis; turbidimetric and nephelometric methods were developed for the analysis of polydisperse systems. Also, ion-pair–based turbidimetric methods were developed [73–75]. With the development of laser technology, first Raman scattering was separated and developed as an independent technique, allowing the analysis of vibrational and rotational states of a molecule.

Resonance light scattering, also known as resonance Rayleigh scattering (RLS), or enhanced Rayleigh scattering, is a simple, rapid and sensitive method for the study of aggregation of molecules. It was first predicted by Placzek in the mid-1930s and later studied as resonance-enhanced Rayleigh scattering (RERS) for diphenylpolyenes [76], for a series of coumarin dyes [77] and for aggregates containing porphyrins [78].

Starting with the 2000s, a series of studies underlined the utility of the method in the assay of pharmaceuticals as ion pairs with organic dyes [79–81] or using a counterion attached to nanoparticles [82, 83] but also for unravelling of their interaction mechanisms with macromolecules of biological interest (transport proteins, DNA) [84–86]. Recent studies have highlighted the potential of this technique to elucidate the action mechanisms of pharmaceutical substances at the molecular level: the mechanism of interaction of oridonin (natural substance with anticancer effect) with DNA macromolecule was revealed [87]; also, the molecular mechanism by which quercetol affects the bioavailability of propranolol was explained [88].

The ion-pair–based assay methods were developed according to the technique proposed by Pasternack et al. [78]. The RLS spectra are registered using a steady-state spectrofluorometer through synchronous scanning of both monochromators. Near or within the range of the absorption band, an enhancement of the scattered signal is observed, which no longer obeys Rayleigh's law. The effect was largely attributed to a scattering-absorption-re-scattering process. For a certain concentration range, increments in the scattering intensity are directly proportional to the concentration of the analyte.

The majority of the substances determined are hydrophilic organic molecules, largely hydrated in water. The ion pairs are formed mostly by an experimentally conducted hydrophobic ion pairing. An increased ionic strength determines the chemical species involved in the ion pairing to become more hydrophobic because the solvent molecules in their hydration shell are attracted in competitive solvation equilibria of the inorganic ions. Generally, the optimum pH and increased ionic strength are obtained using Britton-Robinson buffer. Molecular absorption spectra are used as a previous step in developing RLS methods. By monitoring changes in the absorption spectra, the optimum counterion is selected, and the experimental conditions for quantitative ionic association equilibrium (pH, ionic strength, reaction time) are established. For example, in the case of streptomycin (STR) assay in ionic association with Congo red (CR) [89], for the Britton-Robinson buffer (pH value 5.5), a maximum blue shift (from 497 to 487 nm) and hypochromic effect were obtained, indicating the quantitative formation of the STR-CR ion pair. In these experimental conditions, maximum scattering intensity was obtained (**Figure 3**).

Resonance light scattering methods have many advantages such as great sensitivity and selectivity, simple experimental procedure and the use of accessible equipment (classical spectrofluorimeter) [90], but no validated methods have been published yet. RLS signals suffer from fluctuations caused by many variable factors such as environmental conditions in the reaction medium (pH, ionic strength, temperature and polarity), reagent concentration and the incident light intensity [91].

The challenge for the analysts is to improve the technique in order to obtain reproducible results. Resonance light scattering ratiometry was proposed and applied to the study of the interaction between porphyrins and heparin, in order to solve the problems correlated with the single wavelength measurement. The method provides precise data by taking the intensity ratio at two suitable wavelengths [91].

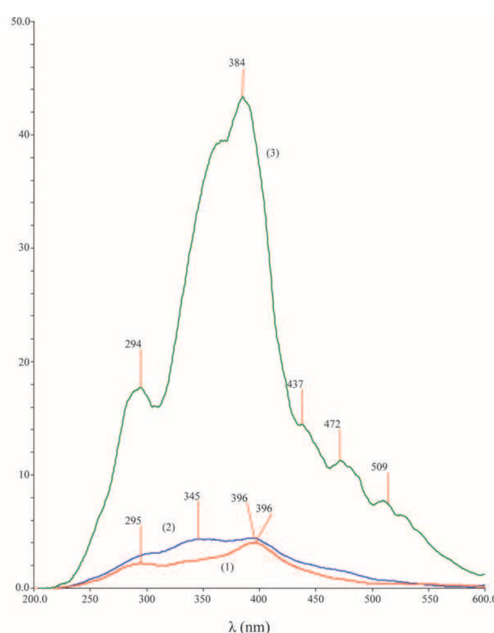

Figure 3. RLS spectra of CR (1), STR (2) and STR-CR ion pair (3) (from Ref. [89], with permission).

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From our experience, slight variations of the ionic strength cause changes in the RLS signal intensity. In routine analysis, it is difficult to obtain identical values of this parameter, and therefore it is difficult to obtain reproducible results. A favourable effect on ion pairing may be obtained by adding small quantities of methanol or ethanol. They have strong water-structuring effect [92, 93], so the hydrophobic interactions for the ion pair can be enforced by engaging water and alcohol molecules in hydrogen bonds, thus dehydrating the substances of interest.

3.4. Challenges and perspectives in IP spectrophotometry

Among the permanent challenges in ion-pair spectrophotometry applied in the pharmaceutical field, one can number the increase of the sensitivity, enabling a more comprehensive study of the mechanisms underlying biochemical processes based on ion-pair equilibrium and finding appropriate conditions to obtain ion pairs for novel pharmacologically active substances.

In terms of increasing the sensitivity of the ion-pair–based methods, the best perspectives are offered by the RLS and fluorimetry, especially when the counterions fixed at the surface of QDs (capped QDs) are used. Using post-column ion pairing, RLS method has been incorporated as a detection technique in high-performance liquid chromatography [94] and capillary electrophoresis [95]. Studies are needed to obtain reproducible results of RLS and to validate the assay methods.

Ion pairing is a fundamental interaction in biological systems. Molecular recognition and protein function are biochemical processes based on ion pairing, and obtaining experimental evidence on the dynamics of macromolecules is a challenge. First experimental data on ion-pair dynamics at protein-DNA interfaces, obtained using nuclear magnetic resonance spectroscopy, were published by Anderson et al. [96].

Polyphenols, an important group of pharmacologically active substances, have not been characterized in terms of the ability to form pairs. Perspectives are opened by recently published study [97], which evaluates the photodynamic therapeutic effect of the curcumin on breast cancer cells using curcumin-methylene blue ion-pair–based nanoparticles. There are numerous substances in this class to be studied.

4. Conclusions

The present work underlined the existence of ion-pair spectrophotometry as a distinct group of methods largely used in the pharmaceutical field. Its evolution was dynamic and was correlated with the elucidation of ion-pair formation mechanisms and the development of computational chemistry. In medicines control, ion-pair molecular absorption spectrometry has the most numerous applications. Generally, organic solvents were used as reaction media. With the development of resonance light scattering techniques, the number of the applications of the ion pairs formed in aqueous solution has increased significantly. Fluorimetry, more sensitive, is also used as an assay method but mostly for biochemical purposes.

If one single feature has to be emphasized, the importance of ion-pair spectrophotometric methods in the pharmaceutical field consists in their versatility. Substances with or without characteristic absorption in UV-Vis range or intrinsic fluorescence, hydrophilic or hydrophobic and organic or inorganic, can be determined as ion pairs in bulk or complex matrices using rapid, sensitive and simple procedures.

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