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A REVIEW STUDY ON CHALLENGES OF ION SPECTROPHOTOMETRY IN PHARMACEUTICAL ANALYSIS

M. Bala Krishna*, P. Navya Sri, M. Nafeesa Begum, V. Anusha, K. Katyayani, Dr. K. Padmalatha

Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada-521108.

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***Corresponding Author**
M. Bala Krishna
Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada-521108.

ABSTRACT

Researches are still focused on the concept of ion pair formation their properties and applications in the various fields which was introduced in the year 1926, by Bjerrum. A pair of oppositely charged ions held together by coulomb attractions without formation of a covalent bond. Experimentally an ion pair behaves as one unit in determining conductivity, kinetic behaviour, osmotic properties, etc. investigation methods used are various, from classical conductometric measurements up-to-date methods such as spectrophotometry, chromatography and capillary electrophoresis. In case of pharmaceutical field, these ion pair are mostly used to develop the methods of separation identification and assay for the active substances in complex matrices, to obtain pharmaceutical formulations with controlled release and to explain the mechanism transport and action for certain drugs. This is an attempt to describe the new trends in spectrophotometry of ion pairs and their applications in the pharmaceutical field. The development of concept and types of ion-pairs are discussed first; further; examples of applications using molecular absorption, fluorimetry and resonance light scattering spectrophotometry are presented. Based on the literature data and the 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.

INTRODUCTION

Ion-pair spectrophotometry refers to analysis methods based on the optical properties of the ion pairs. Infrared, Nuclear Magnetic Resonance and Raman spectrometry these are the

methods used to investigate the structure of ion pairs, molecular & atomic absorption, fluorimetry and resonance light scattering are used as assay method. It is also known as *ionic association or ionic association complexes*, ion pairs are pairs of oppositely charged ions held together by coulomb attractions without formation of covalent bond.[1] The lifetime of an ion-pair was determined to be at least 10^{-5} seconds, equivalent to 10^{8} molecular vibrations, demonstrating that ion pairs can be considered as independent species.[2]

The ionic association in which inclusion of a substance causes changes in the physico-chemical properties of the substance without changing its structure, because an ion pair is electrically neutral and has high lipophilicity compared with free ions.[3] The optimum experimental conditions for a quantitative ion-pair equilibrium(solvent, p^{H}, ionic strength) are easily settled. By selecting the optimum reagent (counterion, ion-pair forming reagent) the selectivity of the method can be increased and in the organic phase the ion pair extraction takes place.

The publications shows that appropriateness in the ion pairing in solving important issues in case of the pharmaceutical field, especially in case of analytical chemistry, biochemistry and pharmaceutical technology. These ion pairs are mostly used in developing new pharmaceutical forms with controlled release, especially for peptides. In this case, one of the main advantage is unmodified pharmaco-toxicological profile of the active substance after ion pairing, because it does not show any changes in its structure. The stability[6] and bioavailability[7,8] of the drug can be improved. Investigation in DNA stability in various matrices,[11] protein determination [12] and synthesis of ion pair receptors based on biological models[13] are important applications in the Biochemistry. There are many methods developed based on the ion-pair formation like gravimetric,[17,18] titrimetric[15,16] electrometric,[19,20] spectrophotometry[21,22] and chromatographic.[23,24]

Ion-pair spectrophotometry method had a dynamic evolution over the time. On one side, this is due to elucidation mechanisms involved in ion-pair formation and on the other side, the synthesis of new pharmacologically active molecules at a very low concentration requires sensitive analysis methods. Among them, less used spectrophotometry techniques, such as resonance light scattering, have found an interesting application when ion pairing was taken into account.

2. Fundamentals of ion pair

2.1. History of ion-pair concepts

The history of ion pair started in the year 1887 with **ARRHENIUS**, who structured the theory of electrolytic dissociation, the ion-pair equilibrium is first considered for the inorganic ions. And the definition was given in the year 1923 by **DEBYE& HUCKEL**. In 1926, **BJERRUM** introduced an association constant in the **DEBYE-HUCKEL** equation and demonstrated that ion-pair equilibrium is dependent on the dielectric constant of the solvent, temperature and 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. And the studies were performed for the elucidation of the existence of the solvent molecules in the ion pair formation.[27] Many of the conductivity measurements were done to confirm the theoretical studies on ion pair formation.

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 can't be considered as rigid and spherical.[28] In 1967, **HIGUCHI et al.,** studied how a contact ion pair can be solvated in various solvents. These ion pairs are formed between 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. The ion pairs formed between two large ions, no solvation is observed.[29]

Diamond,[30] proposed a mechanism of ion-pair formation in aqueous solution by using hydrophobic interactions. The driving force used for the ion-pairing is 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 is strictly electrostatic between two oppositely charged ions, no new electronic bands appear in the absorption spectrum.[28] Spectral changes indicated that, ion

pairs which are formed by the organic ions, additional interactions (aromatic stacking, charge transfer, hydrogen bonding) might exist.

Aromatic stacking is mainly indicated by hypochromic effect in the absorption spectra and it was demonstrated by thermodynamic studies for 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 spectrophotometry by a hypochromic (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]

The main mechanisms involved in the ion pair formation are generally accepted as electrostatic interactions, hydrophobic interactions and also the proton transfer. And the ion pair stability depend upon the structure and size of the ions, on their acid-base and hydrophobic properties and on the solvent nature as well.

The current methods used for the study of the ion-pair equilibrium are spectrophotometry[23,24,36] (molecular absorption, resonance light scattering and fluorescence), conductometry[35], chromatography 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 ionic association equilibrium. Such studies can easily explain the formation of a certain complex, or predict it, and are commonly validated by spectrophotometry methods.

2.2. Types of ion pairs

The structure of ion pairs and the formation mechanisms were established based on the interaction types of the ions in the solution. Considering the solvation, ion pairs exist in the

tight (contact, intimate) form (no solvent molecule is involved in ion pair) and in the loose form (one or more solvent molecules are included in the ion pair). Depending upon the number of the solvent molecules involved, there are 2 types solvent-***sharing*** type (a single solvent molecule is involved) and ***solvent-separated*** ion pairs (when more than one solvent is involved).[1]

Based on the structure of 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 forms). 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 p^H, 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 mechanism of ion pairing. The ion pairs that contain an organic ion are mostly used in the pharmaceutical field.

Based on the solubility of the ion pairs selected is also the reason for classifying the ion pairs. Based on this there are two categories which are 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] And the soluble ion pairs are most numerously used for the application purpose.

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 counter ions.

3. Spectrophotometry applications of ion pairing in pharmaceutical analysis

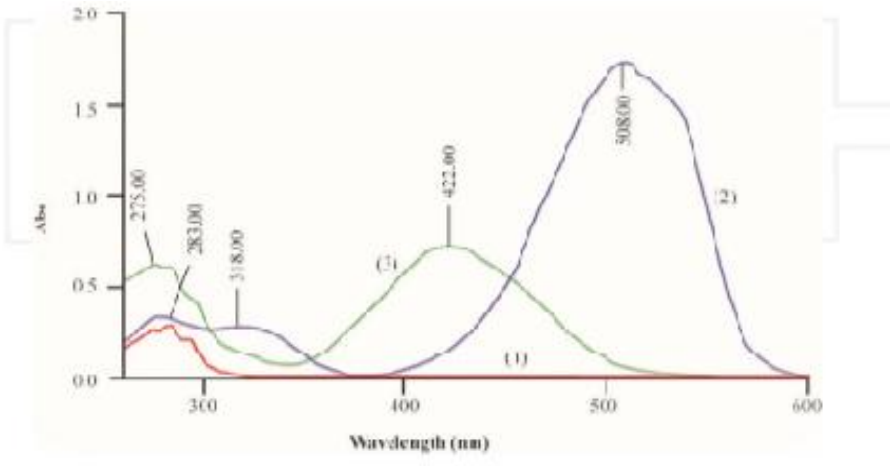
3.1 Molecular absorption spectrophotometry

The most ion-pair spectrophotometry assay methods published in pharmaceutical field are based on UV-V is 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.

The extractive spectrophotometry methods are widely used, the ion pairs are the extracted in an organic solvent, and the extract is further analyzed. For the quantitative extraction in the selected organic solvent, the optimum pH valve, concentration of the reagents and organic strength.

The selection of the counter ion 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 counter ion, 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 spectrophotometric ally by a shift of the absorption peak of the chromosphere. 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 Florae 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. These spectral changes indicate the stabilization of the ion pair by aromatic stacking.[31]

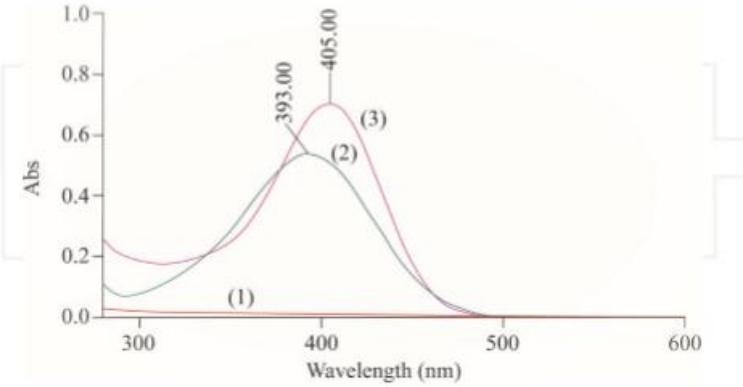


Bromocresol purple (BCP)[43] and alizarin red[44] were also used as counter ions in the assay of TBF using extraction methods.

As counter ions, 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 colored substances.

Hexadecyltrimethyl ammonium bromide was used to develop an extractive spectrophotometry method for the assay of nimesulide by Florae et al.[45] As CTAB is a chain type reagent, with no aromatic rings in the structure and no characteristic spectrum in UV-V is region, NS in its ionized form is the reagent having a peak in visible range. Therefore, when the ion pair is formed it causes a red shift and hyper chromic effect.



Effect Sulfonephthalein dyes, such as Bromocresol green,Bromocresol purple and brilliant blue G,[46] were also used as counter ions 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 be develop non-extractive ion-pair-based spectrophotometry methods in non aqueous 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 results in narrower linearity ranges for the non-extractive compared methods compared with the extractive ones.

Analyte	Method	Counter ion	Solvent	Linearity range
Desloratadine	NE	Eosine	H_2O	0.31-2.81
	E	$[Co(SCN)_4]^{2-}$	$CHCl_3$	0.5-3
Losartan	NE	Eosine	H_2O	2.5-20
	E	Calmagite	$CHCl_3$	10-100
		Orange II	$CHCl_3$	10-100
Levofloxacin	NE	BCG	CH_2Cl_2	1-20
	E	Bromophenol blue	$CHCl_3$	1.85-31.5
		BCG	$CHCl_3$	1.85-25
Amipcillin	NE	Pyrocatechol violet	H_2O	0.2-28
	E	$[Mo(SCN)_6]^-$	CH_2Cl_2	1.5-77.5
Amoxicillin	NE	BCG	$(CH_3)_2SO$	1-13
	E	Methylene blue	$CHCl_3$	3.5-90

3.2. Fluorescence spectroscopy

Among spectrophotometry 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 fluorophores 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 fluorophores (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 structure, if one of the ions is a fluorophores, the counter ion can act as a quencher. For a certain concentration range, the decrease of the fluorescence intensity is proportional with the analyze 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 fluorophores. Organic substances with native fluorescence that can be used as counter ions are few; therefore, there are not many published applications. Literature data on ion-pair fluorescence methods for the assay of pharmaceutical substances are summarized below.

Fluorescent reagent	Analyte
Extractive method	
Erythrosine B	Erythromycin
9,10-Dimethoxyanthrancene-2-sulphonate	Imipramine Desipramine Amitriptyline Nortriptyline Clomipramine Doxepin
Nonextractive Methods	
Eosine	Astemizole Amitriptyline Clomipramine Rosiglitazone Pioglitazone

Fluorescent reagent	Analyte
Eosine (as chelate with pd^{2+})	Ciprofloxacin, Norfloxacin
Safranin T	Meloxican
4,5-Dibromofluescein	Ceftazidime, Cefoperazone
	Albendazole

Berberine, an isoquinoline alkaloid, is a pharmacologically active fluorophores, 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 per chlorates anions.[68] Nanoparticles containing berberine-tetraphenylborate ion pair were prepared, and the cell membrane of cancer cells was studied by Soulie et al.[69]

Lately, the research in Nano science opened an even wider pathway in fluorescence studies involving ion pairing. Quantum dots (QDs) are 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 with different ligands in ionized form. An example is the determination method developed for albendazole, using glutathione-capped cadmium telluride 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 bio molecules in complex biological matrices by flow cytometry[71] and also for kinetic studies using fluorescence microscopy.[72]

3.3. Light scattering spectrometry

The Irish physicist john Tyndall was observed the light scattering in the late 1860s. the theoretical basis of electromagnetic wave interaction with particles than the wavelength was developed by eminent British physicist lord Rayleigh in(1870-1899).now ,the scattering of light by particles in a suspension is accepted to be elastic(without change in the wavelength in 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

In Chemical analysis light scattering method was widely used since from 1950s;for the analysis of polydisperse system turbid metric and nephelometric methods are developed, also ion-pair-based turbid metric method 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 Rayleigh scattering (RLS) OR enhanced Rayleigh scattering also known as resonance light scattering ,is a simple rapid and sensitive method for the study of aggregation of molecules. pleczek was the first person who predicted it in the mid-1930s and later studied as resonance enhanced ray light 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] are using a counter ion 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]

According to the technique proposed by Pasternak et al. The ion-pair based assay methods were developed[78] through synchronous scanning of both monochromators the RLS spectra are registered by wing a study state spectrofluorometer. An enhancement of the scattered signal is obtained by near (or) within the range of absorption band. Which on larger obay's ray leigh's law. The effects was largely attributed to a scattering absorption-re-scattering process for a certain concentration range, increment in the scattering intensity or directly proportional to the concentration of the analyte.

The majority of the substances determined are hydrophilic organic molecules, largely hydrated in water. Hydrophobic ion pairing is mostly formed anion pairing in experimental condition. 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 counter ion 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 (Figure3).

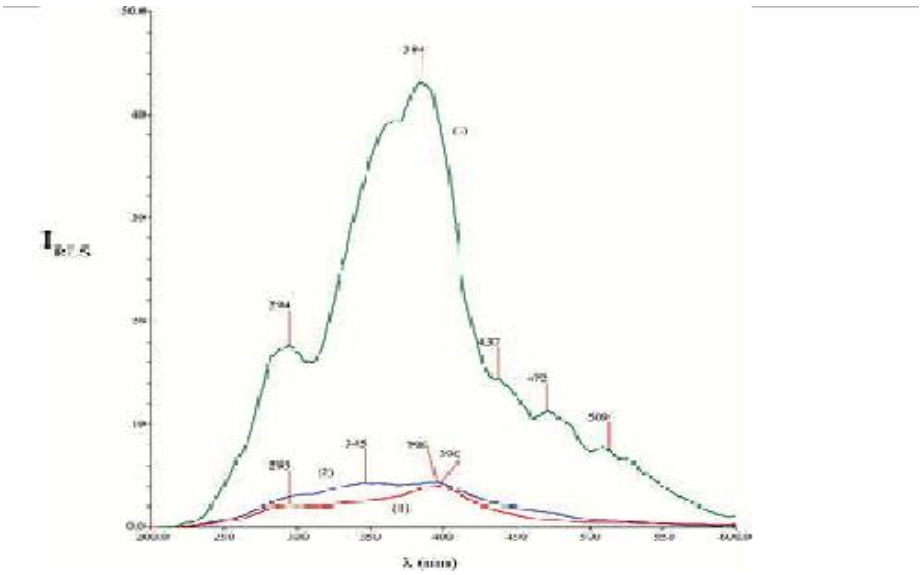


Figure 3.

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

Resonance light scattering methods have many advantages such as great sensitivity and selectivity, simple experimental procedure and the use of accessible equipment (classical spectrofluorometer),[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 radiometry 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]

From our experience slight variation of ionic strength cause changes in the RLS signal intensity. It is difficult to obtain identical values of this parameters in rout ion analysis 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 find 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 counter ions fixed at the surface of QDs (capped QDs) are used. Using post-column ion pairing, in HPLC[94] and capillary electro phoresis[95] the RLS methods was incorporated as an detection technique. Studies are needed to obtain reproducible results of RLS and to validate the assay methods.

In biological system Ion pairing is a fundamental interaction. Molecular recognition and protein function are bio-chemical processes based on ion pairing, and obtaining experimental evidence on the dynamics of macromolecules is a challenge. By using NMR spectroscopy the First experimental data on ion-pair dynamics at protein-DNA interfaces, obtained. Were published by Anderson et al.[96]

Polyphenols are the 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.

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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