Applications of 9-Chloroacridine in Pharmaceuticals

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Abstract :

9-Chloroacridine, a halogenated acridine derivative, has gained significant attention in pharmaceutical research due to its diverse biological activities. This compound serves as a key intermediate in the synthesis of various acridine-based drugs, particularly those with antibacterial, antimalarial, anticancer, and antiviral properties. Its structural framework contributes to DNA intercalation and enzyme inhibition, making it a valuable candidate in chemotherapeutic drug development. Additionally, 9-chloroacridine has been explored for its potential in treating neurodegenerative diseases and as a fluorescent probe in biomedical imaging. Current research focuses on optimizing its pharmacokinetic properties and minimizing toxicity to enhance its therapeutic applications. This review highlights the major pharmaceutical uses of 9-chloroacridine, emphasizing its role in modern drug discovery and development.

Keywords: 9 - Chloroacridine, DNA intercalation , Toxicity, Biomedical imaging.

I. Introduction

Acridines are heterocyclic structures which play an important role in a various diseases; hence they have been extensively used as therapeutics as antibacterial, antiparasitic, antiviral, antiseptic, and anticancer medicines due to their significant role in a number of disorders. Acridines are good candidates as antitumor agents, since they are able to form complexes with DNA. They are capable of interacting with nuclear DNA in a sequence-specific manner and with biological targets, such as topoisomerase I and II and telomerase . The planarity of the polyaromatic ring structure of acridines has been linked to their biological activity. This can intercalate inside double-stranded DNA, causing stacking and interfering with cellular processes. For spectrophotometric analysis, a wide range of colorimetric or chromogenic reagents [1], are available on the market, including the Bratton–Marshall Reagent (N-1-naphthyl ethylene diamine dihydrochloride) (BM reagent), 3-Methyl-2- benzothiazolinone hydrazone hydrochloride (MBTH), Para dimethyl amino benzaldehyde (PDAB), para dimethyl amino cinnamaldehyde (PDAC), Folin–Ciocalteu reagent (FC reagent), 1,2-Naphthoquinone-4-sulfonate sodium (NQS), 2,6-Dichloroquinone chlorimide (GIBB's reagent), Bathophenanthroline, 1,10-phenanthroline, 2,2'-Bipyridine,4-chloroimide 2,6-dichloroquinone, Hydrazine 2,4-Dinitrophenyl, salt of 2,3,5-triphenyl tetrazolium, Molybdenum blue, ninhydrin, phosphomolebdic acid, dimedone, deniges reagent, dyes, froehde reagent, hydroxyl amine, Lucas reagent, Vanillin, 4-hydroxy-3-methoxybenzaldehyde, Simon's reagent, Ema merie-Engel reagent, etc. NQS and MBTH are two examples of chromogenic reagents that can also be utilized as derivatizing agents for spectrofluorimetric analysis [2,3]. The broad use of spectrophotometric techniques in the pharmaceutical sector for drug development, quality assurance and regulatory compliance accounts for their significance [4]. This techniques adaptability is increased by the ability to target particular functional groups or chemical structures of drug molecules with various reagents, which makes it appropriate for a wide range of applications, including impurity profiling and drug assay. It is also essential for routine pharmaceutical examination since spectrophotometry provides a non-destructive method of analysis that preserves samples while yielding useful information on concentration, purity and degradation.

II. Different types of reagents are

A. Complexing agents :

Pharmaceutical analytes and complexing agents combine to generate stable, colorful complexes that increase the analyte's absorbance at a particular wavelength [5]. This makes the spectrophotometric approach more sensitive and makes it possible to quantify the analyte in the sample.

Significance in the Field of Pharmaceuticals : This agents are essential for the detection and measurement of metal ions and medicinal compounds that do not naturally absorb heavily in the UV-visible spectrum, which makes them important in the pharmaceutical industry. They are frequently employed in the assay of medications that contain metals or that have the ability to combine with metal ions, assisting in ensuring the effectiveness and purity of pharmaceutical compositions.

Examples :

1. **Potassium Permanganate :** Potassium permanganate's dual functionality as an oxidizing and complexing agent enables its widespread application in pharmaceutical analysis, encompassing the assay of numerous drugs.

2. **Ferric Chloride :** Ferric chloride is employed as a complexing agent to interact with phenolic moieties in drugs like paracetamol, allowing for their analytical detection.

3. **Ninhydrin :** The ninhydrin reaction is a valuable tool in biochemical analysis, enabling the identification and quantification of amino acids, peptides, and proteins.

B. Oxidizing/reducing agents :

Reducing agents can also alter the oxidation state of the analyte, producing a detectable color shift, while oxidizing agents cause the drug molecule to oxidize, forming a product with distinct absorbance characteristics that are frequently detectable in the visual range.

Significance in the pharmaceutical industry : Drugs without chromophores (light-absorbing groups) require oxidizing and reducing agents. These agents enable precise concentration analysis

of these medications by altering their oxidation state [6]. Since oxidation produces a large number of breakdown products, these reagents are particularly helpful for stability testing.

Examples:

a) Ceric ammonium sulfate is commonly used as an oxidizing agent in the determination of ascorbic acid (vitamin C) and other antioxidants.

b) Sodium thiosulfate acts as a reducing agent in various spectrophotometric methods, especially in the analysis of iodine based reactions.

C. pH Indicators

Compounds that alter color in response to a solution's pH are known as pH indicators. The indicator molecule's dissociation causes a change in color [7] that makes it visible by spectrophotometry by changing its light-absorbing characteristics.

Significance in the pharmaceutical industry : When analyzing medication acid-base equilibria, pH indicators are frequently employed, especially when titrating basic or acidic pharmaceuticals. They are essential for making sure formulations have the proper pH, which might impact the stability, solubility, and bioavailability of drugs.

Examples :

a) In pharmaceutical formulations, bromocresol green is used to test for weak acids.

b) A well-known acid-base indicator for base-forming drug analysis, phenolphthalein changes color at various pH values to show the titration endpoint.

D. Diazotization reagents

Pharmaceutical companies utilize diazotization reagents, usually sodium nitrite and hydrochloric acid, to change primary amines into diazonium salts. Following their coupling with another reagent, these diazonium compounds can create an azo compound, [8] which is typically strongly colored and detectable by spectrophotometry .

Significance in the pharmaceutical industry : The analysis of medications containing primary aromatic amines, which are prevalent functional groups in many pharmaceuticals, benefits greatly from diazotization processes. This technique offers a very sensitive way to measure these

medications in dose and bulk forms. Additionally, it is widely used in quality control and impurity profiling to make sure that pharmaceutical formulations don't contain hazardous byproducts like aniline derivatives.

Examples :

a) Sulfonamide antibiotics : including sulfanilamide, are analyzed using sodium nitrite and hydrochloric acid.

b) To identify medications having amine groups, such procaine, N-(1-naphthyl) ethylenediamine is employed as a coupling reagent in diazotization procedures.

III. Applications of 9-Chloroacridine in Pharmaceutical Field

1. Determination of Sulfonamides and Local Anesthetics by Fluorimetry

Fluorimetric methods analyze pharmaceuticals like sulfonamides and local anesthetics with primary aromatic amino groups by derivatizing the amino group with a reagent, which may or may not be fluorescent, and then measuring the resulting fluorescent product. O-phthaldehyde and 4,5-methylenedioxyphthaldehyde are used to create fluorescent phthalimidine derivatives, detecting sulfonamides in the 10^5 to 10^6 M range. 2,6-diaminopyridine as a fluorescence reagent, achieving sensitivity in the 10^6 to 10^7 M range for sulfanilamide and benzocaine. Typically, these reactions increase fluorescence compared to blank solutions. However, reacting sulfonamides or local anesthetics with 9-chloroacridine produces 9-substituted aminoacridines, resulting in decreased fluorescence relative to the blank acridine solution. This quenching effect [9] is advantageous for analyzing trace amounts of these drugs by measuring the reduction in fluorescence. This method allows drug determination even with a fourfold excess of the acridine reagent .

This technique is used to various sulfonamides and local anesthetics, demonstrating its usefulness in analyzing these drugs in combination with other pharmaceuticals, and included a comparative study with other fluorometric procedures. When 9-chloroacridine reacts with sulfonamides and local anesthetics that have a main aromatic amino group, aminoacridine hydrochlorides are produced. The fluorescence of the 9-chloroacridine reagent solution is quenched as a result of the production of these derivatives. Drugs in the 10^5 to 10^6 M range can be analyzed by tracking the fluorescence at the activation and emission wavelengths of 385 and 420 nm, respectively. The technique was used on a variety of sulfonamides, local anesthetics, and pharmaceutical

combinations that contained these substances [10]. This method was compared with other established fluorometric techniques.

2. An Electrochemical investigation of the redox behavior of 9-chloroacridine and how it interacts with double-stranded DNA

The electrochemical behavior of 9-chloroacridine (9Cl-A), a precursor for synthesizing acridine derivatives with cytostatic activity, is complex, pH-dependent, and governed by diffusion-controlled irreversible processes. Upon oxidation, 9Cl-A forms a cation radical monomer, which subsequently dimerizes and undergoes further oxidation to yield a new cation radical. Reduction of 9Cl-A leads to the generation of radical monomers that stabilize through dimer formation. These phenomena were investigated using cyclic, differential pulse [11], and square wave voltammetry at a glassy carbon electrode

The interaction between 9Cl-A and double-stranded DNA (dsDNA) was examined using a multilayer dsDNA electrochemical biosensor with 9Cl-A concentrations ranging from 1.0 × 10^-7 M (the lowest detectable concentration for DNA interaction) to 1.0 × 10^{-4} M. This analysis allowed for the calculation of a binding constant (K) of 3.45 × 10^{-5} M and a determination of the change in Gibbs free energy for the formed adsorbed complex. The results indicate that complex formation is a spontaneous process, occurring via 9-Cl-A intercalation into dsDNA, which induces structural changes. Molecular docking analysis further supports the intercalation [12] of 9Cl-A into dsDNA.

3. Using 9-Chloroacridine for Colorimetric Determination of Certain Sulfonamides

A colorimetric method leveraging the reaction between 9-chloroacridine and primary aromatic amines has been utilized to analyze various sulfonamides and their mixtures. This technique exhibits sensitivity comparable to other sulfonamide assays, particularly the widely used diazotization-coupling methods. Quantitative analyses indicate that this approach allows for the detection of sulfonamides even in the presence of compounds such as azo dyes, tetracycline hydrochloride, sodium penicillin G, hexamethyleneamine, and other sulfonamides.

This assess a colorimetric analysis method for sulfonamides, based on the reaction between organic amines and 9-chloroacridines, resulting in the formation of highly colored aminoacridine hydrochlorides. This assay procedure, previously reported for primary aromatic amines, has now

been applied to the analysis of several sulfonamides and their mixtures. A comparative evaluation of this technique was performed against the Bratton and Marshall procedure.

4.Salbutamol and Terbutaline Spectrophotometric Determination with the 9-Chloroacridine Reagent

Salbutamol sulphate (SBS), also known as albuterol, is chemically identified as bis [(1RS)-2-[(1,1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxy methyl phenyl] ethanol] sulphate. It functions as a cardiovascular agent and bronchodilator. SBS is commonly used by athletes and is monitored as an anti-doping agent. High doses of SBS may exhibit lipolytic effects, and residues, particularly in the liver and meat, can be toxic to humans.Terbutaline sulphate (TERS) is chemically named 2-t-Butylamino-1-(3,5-dihydroxyphenyl)ethanol sulphate. It is an orally active adrenergic β2 receptor agonist effective as a bronchodilator. TERS is used to treat wheezing and shortness of breath associated with lung conditions such as chronic obstructive pulmonary disease, bronchitis, asthma, and emphysema [13]. It has minimal effect on β1 receptors, thereby reducing direct cardiovascular stimulation.

Various analytical methods have been developed for the quantitative estimation of SBS and TERS in pharmaceutical dosage forms. For SBS, the standard determination method involves potentiometric titration in a non-aqueous medium. Other techniques reported in the literature include High-Performance Liquid Chromatography (HPLC), fluorescence spectroscopy, and voltammetry. TERS has been quantified using methods such as High-Performance Thin-Layer Chromatography (HPTLC), fluorimetry, HPLC, capillary electrophoresis, capillary electrophoresis-mass spectrometry (CE-MS), and voltammetry. However, many of these methods require complex procedures and costly instrumentation.

Spectrophotometric techniques are favored due to their simplicity, sensitivity, and cost-effectiveness. A novel spectrophotometric method has been described for the assay of SBS and TERS in their pure forms and dosage formulations. This method is based on a nucleophilic substitution reaction where the drugs interact with 9-chloroacridine (9-CA) in a basic medium, resulting in products with absorption maxima at 600 nm for SBS and 588 nm for TERS. The method adheres to Beer's law [14] within concentration ranges of 0.5–12.0 µg/mL for SBS and 0.1–6.0 µg/mL for TERS, with molar absorptivity values of 1.913×10^{4} and 1.0755×10^{5} $L\cdot mol^{-1}\cdot cm^{-1}$, respectively. Recovery rates are 101.3% for SBS and 99.6% for TERS, with relative

standard deviations (RSD) below 1.7% for both drugs. Proposed reaction mechanisms have been suggested.

5.Using Quenching Fluorometry to Determine N-Arylhydroxylamines with 9-Chloroacridine

The interaction between N-arylhydroxy lamines and 9-chloroacridine leads to a decrease in the inherent fluorescence of 9-chloroacridine solutions. By monitoring this fluorescence at excitation and emission wavelengths of 400 nm and 430 nm, respectively, arylhydroxylamines can be analyzed at concentrations ranging from 10^6 to 10^5 M with an accuracy of 1-5% [15]. This method has been applied to various arylhydroxylamines and mixtures containing hydroxylamines alongside potential interfering chemical species.

Arylhydroxylamine intermediates have been implicated in the carcinogenesis mediated by certain aromatic amines and are postulated to be proximal carcinogenic species. However, their low steady-state concentrations in chemical and biochemical systems have precluded their rapid detection and quantification. Several methods available for the detection of aromatic hydroxylamines at microgram levels have been recently reviewed and include colorimetric methods [16] and gas chromatographic procedures, which may require derivatization depending on the position of the hydroxylamine group.

6.Aminophenol Isomer Determination spectrophtometrically using 9-Chloroacridine Reagent

Aminophenol isomers—o-aminophenol, m-aminophenol, and p-aminophenol—are primarily used as intermediates in the manufacture of dyes and pigments. They also serve as vulcanization accelerators and antioxidants in rubber compounds. These crystalline solids are of low volatility and can cause contact dermatitis, which appears to be the greatest hazard arising from their use in industry. Additionally, they are the main metabolites of aniline both in vivo and in vitro.A simple, sensitive, and accurate spectrophotometric method [17] has been developed for the quantitative determination of these aminophenol isomers. The method is based on the interaction between these amines and 9-chloroacridine reagent (9-CA).

The spectra of the products show maximum absorption at 436 nm. Beer's law is obeyed in the concentration range of 0.25–12, 0.2–10, and 0.0–10 µg mL^{-1} with molar absorptivity values of 7.20×10^3, 7.67×10^3, and 5.93×10^3 L mol^{-1} cm^{-1} for the above-mentioned isomers, respectively.

The mean percent recoveries range between 97.3% and 101.07% with relative standard deviation (RSD) ≤4.7% for all the isomers. In addition, the stability constant has been determined, and the mechanism is proposed for the 9-CA-aminophenol products .

7. Using the 9-chloroacridine Reagent, a New Spectrophotometric Analysis and Kinetic Analysis of Sulfamethoxazole in Pure and Tablet Formulation

A spectrophotometric method has been developed for analyzing Sulfamethoxazole (SMX) in both pure and dosage forms. This method involves the reaction of SMX with 9-chloroacridine (9-CA) reagent in organic and acidic media, [18] resulting in a yellow product with maximum absorption at 448 nm. Beer's law is applicable within the concentration range of 1–30 µg/mL, with a molar absorptivity of 1.63×10^{4} $L\cdot mol^{-1}\cdot cm^{-1}$, demonstrating good detection and quantification limits. The method exhibits an average recovery of 98.43% and a precision of 0.651. It has been successfully applied to determine SMX in commercial tablet formulations, yielding results consistent with the official method. Thermodynamic studies indicate that the complex formation occurs spontaneously, with physical interactions between SMX and 9-CA, leading to increased order in the system. Kinetic analysis reveals that the reaction follows pseudo-first-order kinetics with respect to SMX. Rate constants at various temperatures and thermodynamic activation functions have been determined. Theoretical parameters calculated using the semi-empirical Austin method (AM1) support the proposed reaction mechanism.

Conclusion :

9-Chloroacridine holds great promise in the pharmaceutical industry due to its broad spectrum of biological activities, including antimicrobial, anticancer, antiviral, and antiparasitic properties. Its ability to interact with DNA and enzymes makes it a valuable scaffold for drug discovery and development. While its potential is evident, further research is required to refine its pharmacological properties, minimize toxicity, and enhance its therapeutic efficacy. Continued exploration of 9-chloroacridine derivatives lead to the development of novel and more effective pharmaceutical agents.

9-Chloroacridine serves as a valuable reagent in the pharmaceutical industry, primarily due to its role in synthesizing biologically active compounds. Its reactivity enables the modification of acridine derivatives, Additionally, it plays a crucial role in chemical reactions involving

nucleophilic substitution and functionalization of acridine-based compounds. While its applications as a reagent continue to expand, further research is needed to explore novel synthetic pathways and improve efficiency in drug design and development.

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