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A REVIEW ON ADVANCES IN PAPER ELECTROPHORESIS METHOD FOR AMOXICILLIN: ENVIRONMENTAL AND CLINICAL APPLICATIONS

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Abstract

Amoxicillin, a widely used broad-spectrum β-lactam antibiotic, has become a major environmental contaminant due to its extensive use and incomplete metabolism, contributing to antimicrobial resistance. Effective monitoring of amoxicillin in clinical, pharmaceutical, and environmental samples requires reliable, sensitive, and affordable analytical techniques. Although chromatographic methods such as HPLC and LC-MS provide high precision, they are often unsuitable for low-resource or field settings because of cost and equipment limitations. Paper electrophoresis, a classical yet evolving technique, has re-emerged as a sustainable, portable, and cost-effective alternative for antibiotic analysis. Its simplicity, minimal reagent consumption, and adaptability make it suitable for on-site applications. This review summarizes the development and principles of paper electrophoresis for amoxicillin determination and explores its evolution into modern portable systems. Recent advancements such as paper-based microfluidic devices (μPADs), smartphone-assisted detection, and hybrid electrophoretic-electrochemical platforms are highlighted. The review also discusses applications in pharmaceutical quality control, therapeutic drug monitoring, and environmental analysis, emphasizing its relevance to green analytical chemistry. Key challenges—including limited sensitivity, lack of standardization, and reproducibility issues—are critically examined, along with future directions integrating artificial intelligence, biosensors, and lab-on-paper systems. Overall, paper electrophoresis represents a promising, eco-friendly analytical approach for sustainable monitoring of amoxicillin and combating environmental antibiotic pollution.

Introduction

1.Overview of Amoxicillin and Its Clinical/Environmental Relevance

Amoxicillin, a frequently prescribed β-lactam antibiotic related to the aminopenicillin class, works by attaching itself to penicillin-binding proteins and suppressing the formation of bacterial cell walls [1]. It continues to be one of the most widely used antibiotics in both human and veterinary medicine and is effective against many different kinds of Gram-positive and Gram-negative bacteria [2]. However, wastewater and agricultural runoff degrade the ecosystem due to its widespread use and incomplete metabolism [3]. Surface waters and sediments have been found to contain amoxicillin residues, which exacerbate ecological imbalance and antimicrobial resistance. Thus, it is essential to create effective analytical instruments for

monitoring amoxicillin in environmental and clinical samples.

2 Importance's of Analytical Techniques for Antibiotic Detection

Accurate detection of antibiotics like amoxicillin is vital for drug quality control, therapeutic monitoring, and environmental risk assessment. Chromatographic methods such as HPLC and LC-MS provide high sensitivity but require costly instruments, trained personnel, and laboratory facilities. Hence, low-cost, portable, and field-compatible methods are increasingly preferred, especially in resource-limited settings. Simple and rapid analytical techniques capable of on-site detection can support surveillance programs and minimize environmental impact [4].

3. Rationale for Using Electrophoretic Separation Methods

Electrophoresis enables the separation of charged molecules under an electric field and is suitable for compounds like amoxicillin that possess ionizable group . Paper electrophoresis offers advantages such as minimal reagent use, simplicity, and adaptability for field applications. Compared with chromatography, it is inexpensive, portable, and environmentally friendly. Modern developments such as paper-based microfluidic devices and smartphone- based detection have improved its sensitivity and applicability for pharmaceutical and environmental analysis [5].

4 Scope and Objectives of the Review

This review aims to comprehensively analyze the historical evolution and recent innovations in paper electrophoresis for the analysis of amoxicillin. Specifically, it (i) outlines the origins and early developments of paper electrophoresis; (ii) reviews analytical principles and influential parameters affecting amoxicillin separation; (iii) compares conventional and modern paper-based electrophoretic systems for pharmaceutical and environmental monitoring; and (iv) discusses the challenges, limitations, and future perspectives of field-compatible paper electrophoretic technologies. The overarching goal is to emphasize how this low-cost, portable methodology contributes to sustainable antibiotic monitoring, supporting global efforts to mitigate antimicrobial resistance and environmental pollution [6].

Historical Background of Paper Electrophoresis

1 Origin and Early Developments:

Paper electrophoresis was first reported by König in 1937, introducing the use of paper soaked in buffer for zone electrophoresis [7].

In the 1940s–1950s, it became widely used to separate proteins and biomolecules due to its simplicity and low cost [8].

2 Classical Techniques for Antibiotic Analysis:

In 1954, Martin and Ames developed methods for protein separation on paper, which later influenced antibiotic analysis.

Early antibiotic analysis relied on the migration of charged molecules under an electric field on paper substrates [9].

Figure 1: Classical Techniques for Antibiotic Analysis

3Limitations of Traditional Methods:

Resolution Issues: Closely related antibiotic compounds were difficult to separate Time-Consuming: Traditional paper electrophoresis required long separation times

Quantitative Challenges: Accurate determination of antibiotic concentrations was limited by non-standardized protocols [10].

2.4 Early Studies on β-Lactam Antibiotic Separation:

In the 1950s–1960s, β-lactam antibiotics (penicillins, cephalosporins) were studied using paper electrophoresis to understand their physicochemical properties [11].Separation was complicated by structural similarities and instability of some β-lactams, which could degrade during electrophoresis [12].

Aim

To review the development and modern advancements of paper electrophoresis methods for detecting amoxicillin in pharmaceutical and environmental samples.

Objectives

1. To outline the historical background and basic principles of paper electrophoresis.
2. To explain the fundamental principles, mechanisms, and factors influencing paper electrophoresis performance.
3. To discuss recent innovations like μPADs and Smartphone-assisted systems.
4. To highlight current applications and future prospects in field-compatible antibiotic analysis.

3. TYPES OF PAPER ELECTROPHORESIS

1. Horizontal Paper Electrophoresis:

Principle: The horizontal paper electrophoresis principle states that the paper strip should lie flat in a buffer solution. At one end of the paper strip, a sample of charged molecule-such as proteins or nucleic acids-is applied. Depending on their size and charge [13, 14], the charged molecules will flow through the paper strip at varying speeds when an electric potential difference is applied across it. As a result, smaller, more charged molecules will pass through the paper with greater efficiency than larger but less charged molecules [15].

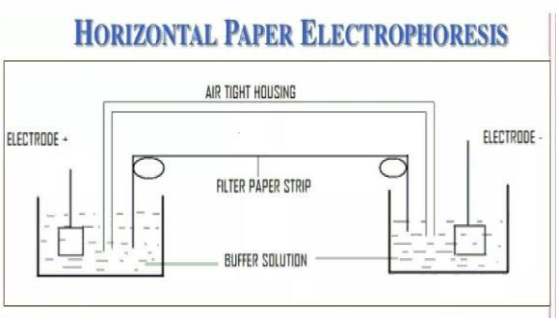

Figure 2: Horizontal Paper Electrophoresis.

Instrumentation:

1. Buffer Tank: The paper strip is immersed in a tank that holds the buffer solution.
2. Power Supply: This is used to apply the electric field required for electrophoretic separation[16].
3. Paper Strip: The separating medium is an absorbent paper strip that performs similarly to filterpaper[17].
4. Sample Application: A tiny line or place on the paper where the sample is applied.
5. Visualization: Following electrophoresis, the separate molecules are seen using specific stains for the compounds being studied [18].

2. Vertical Paper Electrophoresis:

Principle: The working of vertical paper electrophoresis is basically the same as that of horizontal electrophoresis; it differs in that the paper strip is inserted vertically into the buffer solution [19]. With this setup, the separation distance is longer, and sometimes, for larger molecules, better resolution is achieved[20].

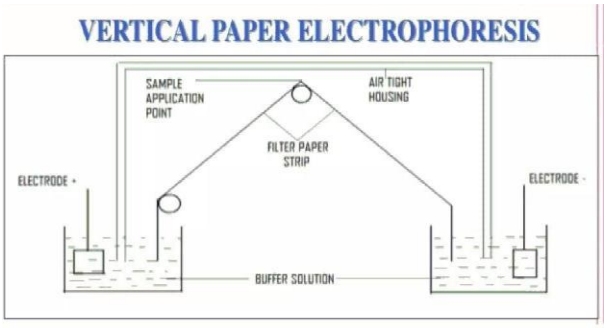

Figure 3: Vertical Paper Electrophoresis.

Instrumentation:

1. Vertical Tank: This is the tall tank into which the paper strip is inserted vertically.
2. Buffer Reservoirs: Two buffer reservoirs, anode and cathode, to be connected through the electrode for establishing the electric field.
3. Power Supply: Supplies the electric current needed to effect separation[21].
4. Paper Strip: An absorbent paper strip, held vertically.
5. Sample Application: Applied at the top of the paper strip.
6. Visualization: Just like horizontal electrophoresis, this is done after the run followed by appropriate visualization stains[22].

3.1 Principle and Mechanism of Paper Electrophoresis

Paper electrophoresis is a classical analytical technique used for the separation of charged biomolecules such as amino acids, peptides, and proteins based on their differential migration in an electric field through a paper medium saturated with an electrolyte buffer. When an electric field is applied, charged molecules migrate toward the electrode of opposite polarity according to their charge-to-mass ratio. The migration velocity is directly proportional to the electric field strength and electrophoretic mobility [23].

The cellulose fibers of the paper act as a support medium, providing a uniform pathway for ionic movement and preventing convective mixing. Migration is influenced by electrostatic forces, frictional resistance, and diffusion processes. Electroosmotic flow (EOF), caused by the movement of buffer ions along the charged cellulose fibers, can affect migration direction and resolution. Adsorption of analytes on cellulose and differences in ionization state due to pH gradients also influence the separation efficiency [24].

Paper Electrophoresis: Mechanism of separation

Differential migration in an electric field

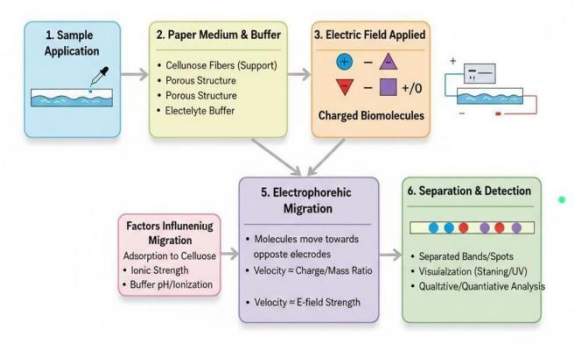

Figure 4: Paper Electrophoresis and Mechanism of separation.

3.2.Fundamental Electrophoretic Theory

The theoretical basis of electrophoresis lies in the motion of charged species under an electric field. The force acting on a charged particle is counterbalanced by viscous drag (), giving rise to electrophoretic mobility , where is the charge, is the viscosity, and is the hydrodynamic radius. Electrophoretic separation depends primarily on the charge and size of analytes. Higher charge and smaller size result in greater mobility. The zeta potential (ζ), which reflects surface charge and surrounding ionic environment, determines the direction and speed of migration
[25].

Resolution between two solutes depends on the difference in their mobilities, diffusion coefficients, and applied voltage. Joule heating (from current flow) increases temperature and diffusion, often decreasing resolution.

Thus, careful control of voltage, temperature, and ionic strength is crucial for efficient separation [26].

3.3. Electrolyte and Buffer Systems Used

Electrolytes provide ions necessary for current conduction and maintain a constant pH, ensuring stability of molecular charge states. Common buffer systems used in paper electrophoresis include: Barbital (Veronal) buffer (pH 8.6): Classical choice for serum protein electrophoresis due to minimal ionic interference and effective pH control.

Phosphate buffer (pH 7.0): Suitable for neutral and weakly acidic biomolecules, maintaining physiological pH [27].

Citrate buffer (pH 6.0): Used for separating acidic proteins and amino acids.

Tris-borate-EDTA (TBE) or Tris-glycine buffers: Provide high conductivity and pH stability for nucleic acid and protein separation.Buffer selection depends on the analyte's isoelectric point (pI), desired pH, and compatibility with detection reagents. Optimal ionic strength ensures adequate conductivity without excessive heat generation. Additives like EDTA or detergents (SDS) are sometimes used to prevent metal ion interference or protein aggregation [28].

3.4. Factors Affecting Migration Rate and Separation Efficiency

Several variables determine migration behavior and overall resolution in paper electrophoresis:

1. pH and Net Charge: The analyte's charge is pH-dependent. Near its isoelectric point (pI), the net charge approaches zero, slowing migration.
2. Size and Shape of Molecules: Smaller and more compact ions move faster than larger or elongated ones of the same charge [29].
3. Electric Field Strength: Higher voltage increases migration speed but may cause Joule heating and band broadening .
4. Buffer Ionic Strength: High ionic strength enhances current but can decrease mobility due to ion shielding and increased heat
5. Temperature: Higher temperature lowers viscosity but increases diffusion and convection currents, reducing resolution[30].
6. Adsorption on Paper Fibers: Interactions between solute and cellulose can cause peak tailing or zone spreading.
7. Electroosmotic Flow (EOF): Movement of the solvent along paper fibers can influence migration direction, especially for neutral species.
8. Sample Load and Application: Large or unevenly applied samples cause broader zones and poor reproducibility [31].

3.5. Paper Matrix Characteristics and Selection Criteria

The paper serves as an inert support holding the buffer and analytes. Its properties directly impact separation efficiency.

Key characteristics include:

Purity: High-purity cellulose (e.g., Whatman No. 1) ensures minimal ionic contaminants that can distort migration pattern .Porosity and Thickness: Determines buffer uptake and capillary action; uniform porosity provides reproducible ion migration [32].

Moisture Content: Paper must be uniformly saturated with buffer for consistent electric conductivity .

Surface Charge: Cellulose fibers possess slight negative charge, contributing to electroosmotic flow; chemical treatment can reduce undesired interactions.

Mechanical Strength: Must withstand handling and post-run staining without tearing or distortion[33].

Selection Criteria: The chosen paper should have appropriate pore size, thickness, and low adsorption capacity for the analyte of interest. Specialized electrophoresis-grade papers (e.g., Whatman 3MM CHR) are preferred for consistent reproducibility [34].

4. Analytical Determination of Amoxicillin

4.1 Structure and physicochemical properties of AMX

Amoxicillin is chemically known as (2S,5R,6R)-6-{[(2R)-2-amino-2-(4-hydroxyphenyl)- acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-24-carboxylic acid, is a β-lactam semisynthetic penicillin from the aminopenicillin class with a broad antibacterial spectrum, used to treat a large number of infections with susceptible Gram-positive and Gram- negative bacteria. It is one of the most frequently prescribed penicillin derivatives within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics (Block, Beale, 2011).AMX is susceptible to degradation by β-lactamase producing bacteria, which are resistant to a narrow spectrum of β-lactam antibiotics, such as natural penicillins. For this reason, it is often combined with clavulanic acid, a β-lactamase inhibitor. The molecular formula of AMX is C16H19N3O5S, available as a white powder [35].

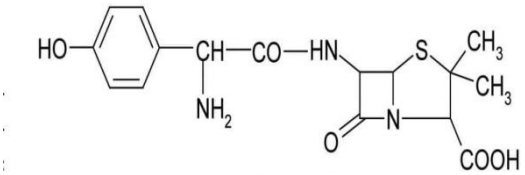

Figure 5: Structure and physicochemical properties of Amoxicillin.

Paper Electrophoresis for Amoxicillin Separation

Working Procedure:

1. **Preparation of Paper Strips:**
   Cut high-quality Whatman No. 1 filter paper into strips, approximately 10 cm in length.
2. **Sample Application:**
   Spot a small volume (1–2 µL) of the Amoxicillin solution onto the center of each paper strip. Allow the spots to dry completely.

3. **Electrophoresis Setup:**
   Place the paper strips in a horizontal electrophoresis chamber filled with an appropriate buffer solution, such as phosphate buffer at pH 7.4.
4. **Application of Electric Field:**
   Connect the electrodes to a power supply and apply a voltage (typically 100–200 V) across the paper strips.
5. **Separation Process:**
   Run the electrophoresis for a specified time (e.g., 30–60 minutes), allowing Amoxicillin to migrate based on its charge and size.
6. **Visualization:**
   After electrophoresis, remove the paper strips and dry them. Stain the strips with an appropriate reagent, such as ninhydrin, to visualize the separated bands.
7. **Analysis:**
   Examine the stained bands under UV light or a suitable imaging system. Measure the distance migrated by Amoxicillin and compare it to known standards for identification [36].

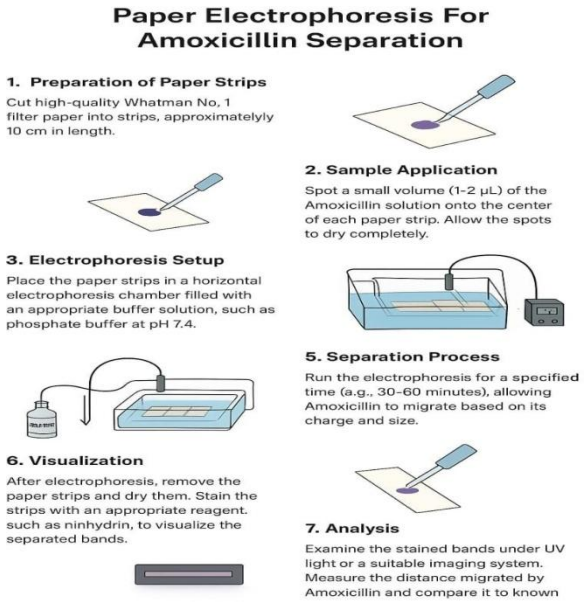

Figure 6: Paper Electrophoresis for Amoxicillin Separation.

Advantages

1. Simplicity and Cost-Effectiveness: Requires minimal equipment and is inexpensive to perform. Portability: Suitable for field studies or settings with limited laboratory infrastructure.
2. Sensitivity: Capable of detecting low concentrations of Amoxicillin.
3. Speed: Provides relatively quick results compared to some other analytical methods.

Disadvantages

1. **Resolution Limitations:** May not effectively separate compounds with similar charge-to-mass ratios.
2. **Quantification Challenges:** Difficult to quantify the exact amount of Amoxicillin without additional techniques.
3. **Environmental Factors:**
   Results can be influenced by ambient temperature and humidity.
4. **Labor-Intensive:**
   Requires manual handling and careful observation, which can be time- consuming.

4.2. Ionization Behavior & Influence on Migration

Because amoxicillin is amphoteric, its net charge changes with pH:

At low pH (< ~2.5), it exists primarily as a cation (amino protonated).

At intermediate pHs (around 6–8), it's predominantly zwitterionic (both positive and negative charges).

At sufficiently high pH (> ~8–9), deprotonation prevails and it becomes net negative.

In electrophoresis, these ionization states dictate direction and rate of migration: cationic at low pH toward the cathode; anionic at high pH toward the anode. The buffer pH thus critically influences mobility, zone shape, and separation from impurities[37].

4.3. Detection Methods

Some commonly used methods:

1. UV-Visible Spectrophotometry: Amoxicillin shows absorption peaks in the ultraviolet region (≈230–272 nm), allowing quantitation in pharmaceutical formulations.
2. High-Performance Liquid Chromatography (HPLC) with UV detection: A highly specific method; reversed-phase columns (C18) and buffers (phosphate or acetate) are common, detecting at ~230 nm.
3. Derivatization + Fluorescence: Since amoxicillin lacks strong native fluorescence, reagents like fluorescamine or dansyl chloride are used to tag it, improving sensitivity in biological / trace analyses.
4. Densitometry after electrophoretic separation: After separating on paper or thin support, scanning densitometry (UV or visible) quantifies zone intensities[38].

4.4 Sample Preparation for Various Matrices

1. **Pharmaceutical formulations**
   Dissolve the dosage form (tablet, capsule) in a suitable solvent or buffer, sonicate or stir, and filter (e.g. 0.45 µm) to remove insoluble excipients. Dilute to working concentration.
2. **Biological fluids (plasma, serum, urine)**
   Precipitate proteins using organic solvents (e.g., acetonitrile, methanol), centrifuge, collect supernatant. Optionally apply solid phase extraction (SPE) (e.g. C18 cartridges) to clean up and concentrate the analyte prior to chromatographic or electrophoretic analysis.

3. **Environmental samples (water, wastewater, soil leachates)**

   Adjust sample pH (commonly to ~4-6), then apply SPE or liquid-liquid extraction (LLE) to concentrate amoxicillin. After concentration/cleanup, evaporate and reconstitute in buffer before analysis [39].

5.Evolution of Paper Electrophoresis Techniques

5.1 Transition from conventional to modern portable systems

Paper electrophoresis began as a simple laboratory separation technique in the early–mid 20th century and was traditionally performed on bench-top rigs using large sheets of filter paper, reservoir buffers and external power supplies. Over the last two decades the format has shifted toward compact, user-friendly devices designed for point-of-need analysis. Key changes include miniaturization of the paper support, low-voltage power sources (battery- or USB- powered), integrated electrode assemblies, and the adoption of disposable cartridges that reduce cross-contamination and operator skill requirements. These adaptations enable rapid on- site separations with minimal reagent consumption and are particularly suitable for environmental screening and basic clinical triage outside conventional labs [40].

5.2 Integration with digital and microfluidic platforms

Modern paper electrophoresis has converged with microfluidic paper-based analytical devices (μPADs) and digital-readout technologies. Patterned hydrophobic barriers and printed channels permit controlled sample routing and multiplexed separations on a single paper device, while embedded microelectrodes and low-current drivers provide reproducible electric fields. Importantly, smartphone cameras and inexpensive optics now serve as primary detectors-converting colorimetric or fluorescence signals into quantitative data via image-processing apps-greatly enhancing portability and data logging. The marriage of μPAD architecture with digital capture and cloud-ready apps has transformed paper electrophoresis from a descriptive laboratory tool into a connected, field-deployable analytical platform [41].

5.3 Advances in visualization and quantification techniques

Visualization methods for paper electrophoresis have progressed beyond classical dyes and staining. Current approaches include enzyme-linked colorimetric assays, nanoparticle- enhanced color changes, fluorescence tags, and contactless electrochemical readouts this is the techniques lower limits of detection while retaining simplicity. In some systems, paper electrophoresis is combined with electrochemical sensors placed downstream of the separation zone to provide selective, sensitive readouts suited to colored or opaque environmental samples[42].

Field-Compatible and Portable Paper Electrophoresis Systems

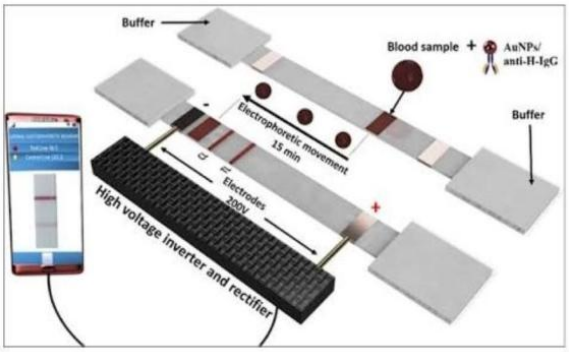

Figure 7: Field-Compatible and Portable Paper Electrophoresis Systems.

6.1 Development of on-site testing devices

Recent efforts have produced compact, user-friendly paper electrophoresis units designed for point-of-need use. Modern on-site devices replace large bench power supplies with battery or USB power, use disposable paper cartridges to avoid cross-contamination, and combine simple sample application with pre-loaded buffer zones to deliver rapid separations in minutes . These systems are tailored for environmental screening (wastewater, surface water) and basic clinical triage where rapid decisions and minimal infrastructure are required [43].

6.2 Miniaturized and paper-based analytical devices (μPADs)

μPADs use patterned hydrophobic barriers and printed channels on cellulose to control fluid flow and perform multiplexed operations (sample splitting, reagent zones, separation lanes) on a single paper substrate . Their capillary-driven transport removes the need for external pumps, dramatically lowers reagent volumes, and allows integration of separation and detection steps on the same device-making μPADs especially suitable for low-resource and field settings [44].

6.3 Integration with smartphone-based detection

Smartphones (high-resolution cameras, processors and connectivity) have become ubiquitous readout tools for paper electrophoresis. After separation, colorimetric, fluorescent or nanoparticle-enhanced signals on paper are imaged and analyzed using apps or cloud software to provide quantitative results, geotagging and remote reporting. Smartphone platforms enable rapid, low-cost quantification and data logging, increasing the utility of paper electrophoresis for distributed environmental surveillance and mobile clinical screening [45].

6.4 Energy-efficient and reagent-less systems for field use

Sustainable field systems minimize power and reagent needs. Strategies include low-voltage circuit designs (microbatteries, USB power), pre-dried reagent zones or hydrogel/ionic-liquid electrolytes that maintain conductivity without frequent buffer replenishment, and solar-assist modules for remote deployment. Such energy-

efficient and low-reagent approaches reduce logistical burden and align with green analytical chemistry principles for field surveillance of antibiotics like amoxicillin [46].

1. Environmental Applications

7.1 Detection of Amoxicillin in wastewater and soil samples

Amoxicillin is frequently detected in wastewater influents, effluents and receiving surface waters, as well as in agricultural soils receiving manure or irrigation with reclaimed water, because a large fraction of the dose is excreted unchanged or as active metabolites. Concentrations reported in wastewater and surface waters vary with geography and season but are often in the ng•L^{-1} to low µg•L^{-1} range; detection in soils is typically lower but persistent, reflecting sorption to organic matter and slower degradation. Routine monitoring relies on validated extraction followed by chromatographic methods (HPLC/UPLC–MS/MS) for reliable quantitation, although low-cost paper- and sensor-based screening methods are increasingly reported for field surveys [47].

7.2 Monitoring antibiotic pollution and resistance hotspots

Environmental monitoring programs use targeted chemical analysis together with molecular assays for antibiotic-resistance genes (ARGs) to map pollution and identify "hotspots" such as pharmaceutical production sites, hospital effluents, and wastewater treatment plants (WWTPs). Wastewater-based epidemiology and integrated surveillance combining antibiotic concentration data and resistome profiling have proven useful for pinpointing sources, tracking temporal trends, and prioritizing interventions. Field-capable screening tools (paper-based sensors, portable electrochemical devices) can extend spatial coverage and rapidly flag locations that require confirmatory lab analysis, enabling more efficient allocation of sampling resources [48].

7.3Role in environmental surveillance and biodegradation studies

Beyond occurrence mapping, environmental studies investigate amoxicillin's fate (sorption, photolysis, biodegradation, and transformation-product formation) because these processes determine persistence and the potential for resistance selection. Biodegradation under aerobic and anaerobic conditions can be slow or incomplete; advanced treatments (AOPs, bioelectrochemical systems, adsorption on activated carbon/biochar) and combined strategies have been developed to increase removal efficiency in WWTPs and contaminated soils [49]. Integrating sensitive monitoring (chemical + ARGs) with targeted remediation research supports risk assessment and informs engineering choices to reduce environmental loads and AMR selection pressure.

2. Clinical and Pharmaceutical Applications

8.1 Determination of Amoxicillin in Biological Fluids (Urine, Plasma, Saliva)

Accurate determination of amoxicillin in biological matrices is crucial for pharmacokinetic studies, therapeutic drug monitoring (TDM), and bioequivalence assessments. Conventional analyses use HPLC-UV or LC–MS/MS due to their high sensitivity and selectivity for quantifying amoxicillin in plasma, urine, and saliva. Plasma concentrations typically range from 1–15 µg/mL after therapeutic dosing, with rapid renal excretion accounting for most elimination. Paper-based electrophoretic and microfluidic systems have recently emerged as portable, low-cost alternatives for point-of-care (POC) drug monitoring, providing near-patient testing capabilities without complex instrumentation. These methods are particularly advantageous in pediatric and geriatric settings where sample volumes are limited [50].

8.2 Rapid Screening for Dosage Accuracy and Drug Stability

Amoxicillin's β-lactam ring is susceptible to hydrolysis and degradation under heat, humidity, and extreme pH, resulting in potency loss. Therefore, stability testing and dosage verification are vital for ensuring therapeutic efficacy. Paper electrophoresis and colorimetric assays provide simple and rapid tools for detecting degraded products and verifying dose uniformity in field conditions. The integration of portable devices allows healthcare providers to perform stability testing in remote regions or during clinical trials without extensive laboratory infrastructure. Additionally, coupling electrophoretic systems with smartphone-based detection enhances result accuracy and enables data archiving for regulatory compliance [51].

8.3 Application in Quality Control of Pharmaceutical Formulations

Quality control of amoxicillin formulations (capsules, suspensions, injectables) ensures compliance with pharmacopeial standards such as those in the United States Pharmacopeia (USP) and European Pharmacopoeia. Traditional chromatographic assays (HPLC, UPLC) remain the gold standard for assessing purity, assay content, and degradation products. These techniques require minimal reagents and can be integrated with digital image analysis for semi- quantitative evaluation, supporting sustainable pharmaceutical quality management [52].

9. Recent Innovations and Emerging Trends

9.1 Green and Sustainable Electrophoretic Techniques

Recent advances in electrophoretic technology emphasize eco-friendly and sustainable analytical practices. Miniaturized platforms such as capillary electrophoresis (CE) and paper- based electrophoresis require minimal sample and reagent volumes, reducing both waste and energy consumption. Greenness metrics—including solvent usage, toxicity, and energy demand—are

increasingly applied to evaluate analytical methods. Studies highlight the use of biodegradable substrates, aqueous buffer systems, and solvent-free sample preparation to align with the principles of green analytical chemistry. These sustainable approaches significantly lower the environmental impact compared with conventional electrophoresis [53].

9.2Hybrid Paper–Microfluidic and Electrochemical Detection Systems

Hybrid microfluidic paper-based analytical devices (μPADs) are merging electrophoretic separation with electrochemical detection to enable compact, disposable, and low-cost platforms. These devices combine the fluid-wicking capability of paper with integrated electrodes, facilitating simultaneous separation and detection on a single platform. Advances in electrode fabrication-using screen printing, carbon inks, and nanomaterial coatings—-enhance sensitivity and reproducibility while allowing multiplexed analyte detection. As a result, hybrid μPAD-electrochemical systems are gaining attention for pharmaceutical, clinical, and environmental applications due to their low reagent use and rapid response [54].

9.3Automation, AI-Assisted Data Analysis, and Portable Instrumentation

The integration of artificial intelligence (AI) and machine learning (ML) has revolutionized electrophoresis data analysis. Automated tools can detect and quantify electrophoretic bands with higher accuracy and speed than manual methods. AI-based gel analysis systems minimize operator bias and improve reproducibility in biomedical and pharmaceutical assays. Additionally, smartphone-based and portable CE devices equipped with contactless conductivity detection enable on-site analyses, transmitting data directly to AI-powered cloud platforms for real-time interpretation. These systems mark a significant step toward fully automated, digital, and field-ready analytical instrumentation[55].

9.4 Case Studies and Comparative Performance Evaluations

Recent comparative evaluations highlight the efficiency of sustainable and hybrid electrophoretic systems:

A smartphone-integrated portable CE device demonstrated comparable separation efficiency to laboratory CE, achieving rapid ion detection with minimal reagent consumption.

Paper-microfluidic electrochemical immunosensors for biomarkers such as prostate-specific antigen (PSA) showed clinically relevant sensitivity and shorter analysis time compared with conventional lateral flow assays. Comparative studies between capillary electrophoresis and HPLC reveal that CE offers superior eco-efficiency and faster analysis for charged compounds, though HPLC still provides better sensitivity for neutral molecules [56].

10. Challenges and Future Perspectives

1. Analytical and Operational Limitations

Despite major technological advances, electrophoretic techniques still face several analytical and operational constraints. Limitations such as low sensitivity for non-ionic analytes, sample adsorption on substrates, and poor resolution in complex biological matrices restrict wider clinical use. Paper-based systems may suffer from inconsistent flow rates, sample diffusion, and limited quantitative accuracy due to paper heterogeneity. Furthermore, electrolyte evaporation, buffer depletion, and limited detection selectivity in portable setups can affect reproducibility and long-term stability. Continuous improvements in substrate quality, buffer chemistry, and miniaturized detection modules are essential to overcome these barriers [57].

2. Standardization and Reproducibility Issues

A significant challenge in electrophoretic device development is the lack of global standardization protocols for fabrication, calibration, and validation. Variations in paper porosity, channel geometry, and electrode placement often lead to device-to-device variability, complicating cross-study comparisons. Reproducibility of analytical performance remains a key concern in microfluidic paper devices and portable CE instruments, especially under varying environmental conditions such as humidity and temperature. Establishing ISO- compatible validation guidelines and adopting automated fabrication could enhance inter- laboratory consistency and promote regulatory acceptance[58].

3.Future Directions in Portable Electrophoretic Diagnostics

The future of electrophoretic diagnostics lies in portable, miniaturized, and smartphone- integrated systems capable of real-time data acquisition and analysis. Advances in contactless conductivity detection (C^4D), fluorescence mini-detectors, and AI-driven image analytics will improve sensitivity and diagnostic accuracy. Integration with cloud-based data management can allow remote access and telemedicine applications, enabling point-of-care testing in resource-limited settings. Moreover, battery-operated or solar-powered CE platforms are being explored to expand electrophoresis applications in environmental monitoring and field diagnostics.

4.Potential Integration with Biosensing and Lab-on-Paper Technologies

Combining electrophoresis with biosensors and lab-on-paper architectures represents a promising direction toward multi-analyte detection systems. Hybrid paper–biosensor platforms can integrate enzyme, DNA, or antibody recognition elements with electrophoretic separation for specific and sensitive assays. These systems have potential in pathogen detection pharmaceutical residue analysis, and environmental toxin screening. Further development in nanomaterial-based electrodes and 3D paper microfluidics may enable full automation,

on-chip reagent storage, and quantitative multi-target analysis. The convergence of electrophoresis, biosensing, and microfluidics could lead to next-generation lab-on-paper diagnostic devices suitable for personalized and decentralized healthcare [59].

Conclusion:

Paper electrophoresis has evolved from a simple laboratory method into a modern, field- deployable analytical tool for antibiotic monitoring. Its low cost, portability, and eco-friendly nature make it particularly suitable for on-site pharmaceutical and environmental applications. Continued integration with digital, microfluidic, and biosensing technologies will enhance sensitivity reproducibility, and automation, supporting sustainable global antibiotic surveillance.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Not Applicable

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