Metallodrug research and analysis using capillary electrophoresis

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We review current and emerging capabilities of capillary electrophoresis (CE) as employed for metallodrug development. The application of gentle separation schemes and successful combinations with element-specific and molecule-specific mass-spectrometric detectors have increased the importance of CE as a tool in this area of biochemical speciation analysis.

This article highlights recent progress of the method in assessing drug stability, separating metallodrugs and their metabolites, characterizing related metal-bioligand species, describing the kinetics and equilibrium of the corresponding metabolic processes, and analyzing biofluids.

Finally, we discuss strategies for developing CE methods in metabolomic and proteomic analysis, centered on metal-based drugs so as ultimately to facilitate their discovery and preclinical investigation.

Keywords: Anti-cancer drug; Biological analysis; Biospeciation; Chiral separation; Diagnostic agent; Metabolomics; Metalloproteomics

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1. Introduction

Metal compounds, particularly metal complexes, are steadily growing in importance as therapeutic drugs [1]. A great many diseases (e.g., anemia (iron), arthritis and asthma (gold), bipolar disorder (lithium), diabetes (vanadium), stroke (magnesium) and ulcer (bismuth)) can all be treated successfully by metal-based drugs. Perhaps the most prominent use of drugs containing a metal center is chemotherapy, where platinum(II) antineoplastic agents are currently applied as frequently as organic drugs, and show superior efficacy for a number of malignancies. Considerable effort is being devoted to developing novel anti-cancer drugs, both platinum and other metal complexes (ruthenium, gallium, titanium), with greater efficacy and reduced toxic side effects [2,3]. Contemporary medicine also greatly relies on metal-based diagnostic agents exploited in magnetic resonance (gadolinium contrast agents) and radioisotope (e.g., cobalt and technetium) imaging. Similarly, radiopharmaceuticals can be employed to deliver sterilizing radiation to targeted sites (e.g., small tumors) in the body. In addition, some complexes (copper, zinc) find medicinal application due to their bacteriostatic, fungicidal or antiphlogistic activity.

It is widely accepted that the process of creating new medicines, including metallodrugs, is far from efficient. The most commonly cited estimate suggests that to launch one new drug, a drug company spends $800 m and the average pharmaceutical development takes more than a decade (i.e. from the time research begins until approval is received to market the drug). It costs so much and takes so long because of the complexity of discovering, developing, testing, marketing and monitoring a new drug. All this implies that there is serious lack of productivity in R&D. Needless to say, to accelerate the drug discovery and development process and reduce the enormous costs and failure rates, pharmaceutical and biotechnology companies particularly have to improve the arsenal of analytical techniques in use. The great complexity of relevant model and real-world samples, which, for metal-based drugs, comprise a great variety of inorganic, organic, biological, and mixed-ligand metal forms, resulting from numerous metabolic transformations and frequent ligand-exchange reactions, puts special requirements on suitable analytical methodologies.

The principal requirement is the ability to differentiate and to measure distinct metal species of interest with minor impact on their speciation during analysis and high tolerance to complex biomatrices. This very advantage of capillary electrophoresis (CE) makes it the method of choice for metal-speciation analysis, as evidenced by a number of excellent reviews [4–6].

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This has also increased the acceptance of CE in therapeutic metallodrug monitoring [7,8].

In such applications, CE offers several advantages over HPLC techniques, having proved itself in the field [9,10]. These merits include: ease of operation; minute sample volumes; lack of a need for (environmentally undesirable) organic solvents; better compatibility with physiological conditions; and, most importantly, impressive resolution of complex mixtures of multiple metal-complexed forms often oppositely charged. In addition, analysis times are generally shorter, so the effect of possible changes in metal speciation in a CE system is kept to a minimum and that makes the technique of benefit in assaying reaction kinetics. Last, but not least, over 50% of the CE instruments worldwide are located in pharmaceutical companies and, with commercial on-line couplings to mass spectrometric (MS) detectors available for species-selective analysis, this could support metallodrug developers.

The purpose of this review is to provide a state-of-the-art picture of the utility of CE techniques for metabolomics, and studying the mode of action and monitoring of metal complexes of pharmaceutical importance, including both approved and promising prototype drugs as well as diagnostic agents. Given our particular research interest, we will place special emphasis on recent achievements of CE in various applications in anticancer metallodrug discovery and development. We also intend to describe hybrid (coupled) approaches that are being used to help address major challenges and further progress of CE in this area.

2. Metallotherapeutic drugs

2.1. Monitoring the stability of metallodrugs

No matter which administration route is appropriate, as soon as a metal-based drug encounters body-fluid circumstances, it will participate in numerous metabolic processes, and inevitably hydrolysis, which is one of in vivo transformation steps. An inherent and usually adverse feature of every (even thermodynamically stable) metal complex, hydrolytic decomposition may still occur in formulations and pose limitations on the clinical application of a metallodrug. Moreover, metabolic degradation via hydrolysis could lead to severe side effects, as in cisplatin chemotherapy [3]. However, the aquated species may play an active role for certain metal-based drugs, affecting their binding to nuclear targets [2,3]. Assessing survival rates is therefore a basic requirement in a systematic metallodrug-discovery process, and CE provides a rapid, cost-efficient, high-throughput and reliable screening method for such measurements. Indeed, the intact drug and its hydrolytic metabolites can be well resolved by exploiting differences in their charge-to-size ratios. Moreover, the time-dependent stability behavior may be assessed quite straightforwardly by means of relative peak-area measurements. These options were demonstrated by profiling the hydrolysis patterns and monitoring the hydrolysis kinetics for several tumor-inhibiting platinum(II) [11–13], ruthenium(III) [14,15], and titanium(IV) [16] compounds under simulated physiological conditions. Specifically, a single-run separation of cisplatin, cis-$[PtCl_2(NH_3)_2]$, $[PtCl(NH_3)_2(H_2O)]^+$, and $[Pt(NH_3)_2(H_2O)_2]^{2+}$ was attained using simple zone-electrophoretic [12] and micellar electrolytes [11], and the primary hydrolysis product, cis-diammineaquachloroplatinum(II), was quantified in human serum [17] (see Section 2.4 for more detail). The stability differences of zinc(II) mixed-ligand complexes with promised bacteriostatic activity were characterized by CE in terms of half-lives and rate constants of the aqueous decomposition reaction [18]; this is most probably a pioneering stability-indicating CE assay for metal complexes.

Redox transformation is another possible pathway of the extracellular metabolism of a drug. However, prior to binding to biological molecules, a transition metal-based drug may be reduced in the presence of pertinent blood-reducing agents (e.g., ascorbic acid and glutathione). This was, for instance, proved by CE monitoring of the conversion of a ruthenium(III) drug candidate into, presumably, the respective Ru(II) species under the action of ascorbic acid [8].

2.2. Separation of chiral drugs

Metallodrugs with asymmetric ligands may exist as diastereomeric mixtures possibly associated with differing isomeric pharmacological activity. An emerging demand for enantiomerically pure drugs to respond to the present regulations of drug-administration bodies increases the profile of stereospecifically evaluating the enantiomers of a drug, to which CE is highly suited [19].

Among the few instances in which chiral metal complexes have been separated by CE are the experiments of Vogt and Werner [20] and Wenclawiak and Wollmann [11], who used sodium dodecyl sulfate micelles via micellar electrokinetic chromatography (MEKC) to resolve a racemic mixture of diastereomers of lobaplatin, an approved anti-cancer platinum(II)-based drug. More recently, our laboratory has demonstrated separations of diastereomers of cytotoxic oxaliplatin derivatives and quantification of their diastereomeric ratios using microemulsion EKC mode [21]. Furthermore, a linear correlation was established between the capacity factors and the corresponding octanol-water partition coefficients that represent a rational estimate of the lipophilicity of a drug. Fig. 1 shows a typical chromatogram for oxaliplatin complexes that provides a basis for fast evaluation of drug nominees regarding the ability to penetrate the cell membrane. It is worthwhile noting that, along with chemical stability, absorption is one of