Cancer is one of the major causes of death in the western world. Current treatment of cancer is limited to surgery, radiotherapy, and the use of cytotoxic agents, despite their well-known side effects and problems associated with the development of resistance. For most forms of disseminated cancer, however, no curative therapy is available and the discovery and development of novel, active chemotherapeutic agents is desperately needed.

An active drug substance is very rarely administered as the pure chemical compound itself. It is almost always transformed or formulated into a well-defined pharmaceutical product, which is suitable for the intended use in humans [1,2]. Thus, pharmaceutical formulation links the preclinical and clinical development phases of an investigational cytotoxic agent by enabling administration and thus evaluation of the drug in humans. Anticancer agents for experimental use are generally intended for intravenous administration to ensure absolute bioavailability, to circumvent possible presystemic metabolism or degradation in the gastrointestinal tract, and to be able to stop administration in case of acute toxicity [1]. Consequently, the development of a pharmaceutical formulation of a novel anticancer agent is associated with the design of sterile and stable injectable products. The pharmaceutical development of a novel compound for human use comprises a range of activities, as is depicted in Figure 1 (adapted from [1]). In order to successfully formulate a novel compound, a broad knowledge of analytical techniques, pharmaceutical sciences, regulatory guidelines, and clinical practice is required [1,2].

![Figure 1](image)

**Figure 1.** Development route of an investigational anticancer agent and pharmaceutical formulation aspects.
Cisplatin, or *cis*-diaminedichloroplatinum(II) (Figure 2) is an anticancer agent that has been in use for over 30 years. Its use is widespread and few other anticancer agents have been proven as effective as cisplatin. However, serious side effects, the most prominent of which are nefro-, oto- and neurotoxicity and severe nausea and vomiting, limit its clinical use. Another major clinical problem is tumor resistance, which can be either intrinsic or acquired [3-6].

![Figure 2. Structure of cisplatin.](image)

The limitations of cisplatin as an anticancer agent have stimulated the search for alternative metal-based anticancer agents and alternative pharmaceutical formulations of platinum, with more acceptable toxicity profiles, but retention, and if possible expansion, of efficacy. Common characteristics of metal complexes, which can prove important in formulation development, are the generally low aqueous solubility and high susceptibility to ligand exchange, resulting in low stability, especially in solution. Different aspects related to the pharmaceutical development of metal-based compounds (depicted in Figure 1) are summarized in this preface.

**Analytical aspects**

For each investigational drug a set of analytical methods has to be developed and validated to enable characterization and quality control of the chemical substance and pharmaceutical product [1,2]. Requirements and specifications for analytical methods, analysis of drug substances and final products have been recorded in regulatory guidelines [7-10]. After definition of a reference standard to which all other batches can be compared, specifications can be defined for both drug substance and final product [2,11].

Structural and analytical characterization and purity determinations are performed by various techniques, such as nuclear magnetic resonance (NMR), infrared (IR), and mass spectroscopy (MS), ultraviolet/visible light (UV/VIS) spectrophotometry, and high performance liquid or gas chromatography (HPLC, GC). The development of a stability-indicating assay, which can discriminate between the parent compound and degradation products is crucial. The method should also be capable of separating the parent compound from contaminants (intermediate
products, solvents used in the synthesis, related products, etc.) and excipients [1,2]. Metals can be analyzed by atomic absorption spectrometry (AAS). This type of analysis, however, only determines the presence of the element and not the form in which it is present or which ligands are attached to it. Therefore, other methods are required to obtain more specific structural information about the compound. A wide variety of HPLC methods has been developed for the marketed anticancer platinum compounds (cisplatin, carboplatin, and oxaliplatin) and their degradation, biotransformation and reaction (with e.g. thiol compounds) products (comprehensively reviewed in [12]). However, development of analytical techniques that provide sufficient insight into the structure and stability of novel metal compounds remains a challenge. This is illustrated by the fact that it was necessary to develop a broad range of analytical techniques to structurally and analytically characterize AP 5280, a novel polymer-conjugated platinum agent [13]. No single method provided a total picture of the structure and integrity of this molecule, but a combination of analytical techniques led to insight into all aspects considered important for its quality control.

NAMI-A, a novel antimitastatic ruthenium agent, is a typical example of a labile metal compound in which ligands are readily exchanged. Many different substitution reactions lead to a plethora of possible degradation products, including products resulting from the oxidation or reduction of the central ruthenium atom. A stability-indicating HPLC method was developed that was capable of detecting the major degradation products [14].

**Solubility aspects**

A drug intended for intravenous administration has to show aqueous solubility in order to prevent precipitation in the blood stream, resulting in pain, phlebitis, and altered bioavailability [1]. A number of solubilization approaches is available for the development of a parenteral dosage form of a drug [2,15]. In summary, these consist of pH adjustment in case of an ionizable functional group, use of co-solvents, surfactants, complexing agents (e.g. cyclodextrins), and/or production of dispersed systems (e.g. emulsions, liposomes, nanoparticles). However, most of these approaches do not improve the solubility of (heavy) metal complexes, although microsomes and liposomes have been successfully developed for cisplatin [3-6]. In fact, many promising novel metal-containing compounds have not even reached the animal toxicology studies, because they could not be dissolved or pharmaceutically formulated. For example, many ruthenium agents containing solely amine and chloride or carboxylate ligands, as well as some of their rhodium counterparts, such as
fac-[RuCl$_3$(NH$_3$)$_3$] and mer-[RhCl$_3$(NH$_3$)$_3$], showed antitumor activity, but proved to be insoluble in aqueous solutions. As a result, they could not be formulated and were abandoned for further development [16].

Very few metals are soluble in water in their elemental state [17]. However, different ligands can provide varying degrees of solubility. Furthermore, conjugation to a variety of compounds such as polymers can increase aqueous solubility of metal complexes, as is the case for AP 5280.

**Stability aspects**

Metals are prone to oxidation and reduction and show facile substitution of ligands, especially in solution. Metal complexes are often more sensitive towards solvents, humidity, light, or air than organic pharmaceuticals. Hydrolytic instability and a wide variety of decomposition products hamper clinical application [5]. At the same time, the reactivity of metal complexes determines their interaction with biological targets (DNA and proteins). These processes are closely related to their mechanisms of action in terms of anti-tumor activity, toxicity and biotransformation [18]. A very reactive compound can decompose before it is applied to the patient and reaches its target, while very stable compounds might be excreted, without any interaction in the biological medium, as is known, for example, of gadolinium complexes [16]. The ligands of metal complexes will determine to a large extent the stability, and thereby the activity, toxicity, and pharmacokinetics of the compounds.

From a pharmaceutical point of view, stability refers to the storage life or utility time, i.e. the period of time a drug substance, excipient, final product, reconstituted product, or infusion solution conforms to its specifications under certain conditions (temperature, humidity, light) [1]. The aim of the pharmaceutical development of a drug candidate is to manufacture a product that has a sufficient shelf-life (>1 year) at a practically convenient storage condition (room temperature (+20-25°C) or refrigerated condition (+2-8°C)). Insight into the stability of a new drug substance, both in the solid state and in solution, is obtained in the preformulation stage, and based on these data along with results obtained in solubility experiments, a decision is made on the formulation of the final product. Accelerated stability studies performed at elevated temperatures will generate data on the stability of drug substance and final product, necessary to designate long-term storage conditions. Real-time stability studies of the pharmaceutical product at the long-term storage condition (set temperature and level of humidity) are then required. Furthermore, to anticipate fluctuations in conditions that could
occur during *e.g.* shipment, stability testing of the final product needs to be performed at
defined intermediate and/or accelerated conditions [19]. Additionally, photostability testing
of the drug substance and final product is required by separate testing [20].

Once a drug substance and final product have been assessed for their stability, it is also
necessary to evaluate the clinical product. In general, a (lyophilized) pharmaceutical product
will be reconstituted and/or further diluted using a suitable (isotonic) infusion fluid to the
desired infusion concentration. Drug stability in infusion solutions can vary widely with the
dilution solution and concentration. For example, the stability of cisplatin in solution is
dependent on the chloride ion concentration, with a higher chloride ion concentration
producing more stable cisplatin solutions [21]. To assure the quality of the product that is
administered to the patient, stability should be assessed for commonly applied infusion
solutions (normal saline, 5% (w/v) dextrose) over the concentration range and under the
conditions (temperature, light) intended in the clinical setting.

**Manufacturing aspects**

Manufacturing of investigational anticancer agents to be used in clinical trials, although
generally performed on a small scale, has to comply with the principles of current Good
Manufacturing Practices (cGMP) [22]. This implies selection, definition, and validation of the
manufacturing process and its subsequent steps. For example, in case of the production of a
sterile dosage form for intravenous use, the stability of the active substance dictates whether
the product can be terminally sterilized by moist heat, or needs aseptic processing and sterile
filtration [23,24]. An aseptic manufacturing process requires well-trained personnel and high
standard environmental conditions of the production facilities, especially with respect to
particles and micro-organisms. Equipment and production materials, such as filters, have to be
validated for their suitability in the manufacture of the specific pharmaceutical product.
Definition of the critical steps in the production process (*e.g.* stability of the formulation
solution, freeze-drying parameters) at the development stage is of crucial importance in view
of future upscaling of the production.

**Compatibility aspects**

During the formulation studies, compatibility of the drug product with the primary packaging
material (container, closure) must be assessed. Furthermore, compatibility testing is important
to determine the optimal administration parameters and devices (container and tubing). These
tests will determine whether *e.g.* adsorption or absorption to the infusion container, tubing, and/or in-line filter, leaching of plasticizers, or physical reactions such as precipitation take place [25]. The platinum compounds cisplatin and carboplatin are known to interact with aluminum, resulting in a black precipitate upon contact, and should not come into contact with aluminum-containing devices [21]. Additionally, *in vitro* biocompatibility testing may provide information about the potential to cause pain, phlebitis, and/or hemolysis of a pharmaceutical formulation. Such reactions can be expected upon intravenous administration of solutions containing drugs with deviant tonicity and pH values from blood [25-27]. Formulation- and administration-related adverse effects can thus, to some extent, be anticipated preclinically and patient discomfort can be minimized by adjustment of administration parameters.

**Scope of the thesis**

In this thesis, the pharmaceutical development of two novel metal-based antitumor agents is presented. Chapter 1 covers the pharmaceutical development of NAMI-A, a ruthenium-based antimetastatic agent. The pharmaceutical development of AP 5280, a polymer-conjugated platinum agent, is described in Chapter 2. The aim of the studies was to develop a stable, intravenous dosage form for both novel agents that would allow clinical evaluation of the products. This comprised development of analytical methods for the quality control of drug substance and final product, the formulation and manufacturing processes, and stability and (bio-)compatibility studies of the final product.

**References**