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RESEARCH PAPER

Pharmaceutical Development of a Parenteral Lyophilized Formulation of the Investigational Polymer-Conjugated Platinum Anticancer Agent AP 5280

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ABSTRACT

AP 5280 is a novel polymer-conjugated platinum anticancer agent showing promising in vitro and in vivo activity against solid tumors. The aim of this study was to develop a parenteral pharmaceutical dosage form for phase I clinical trials. AP 5280 drug substance was characterized by using a wide range of analytical techniques and showed excellent solubility in water. However, as aqueous solutions of AP 5280 proved to be labile upon sterilization by moist heat, it was decided to develop a lyophilized dosage form. Initially, glass vials were used as primary packaging, but this led to a high breakage rate, which could be completely prevented by the use of CZ® resin vials. Stability studies to date show that the lyophilized product in glass vials is stable for at least 12 months when stored at 2–8°C in the dark and the lyophilized product in CZ resin vials is stable for at least 6 months under these conditions. Photostability testing revealed photolability of AP 5280 drug substance and lyophilized product in both types of primary container, necessitating storage in the dark. The first clinical experiences indicate that the proposed formulation is fully applicable for use in the clinical setting.

Key Words: AP 5280; Formulation; Polymer.

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INTRODUCTION

AP 5280 [poly(*N*-(2-hydroxypropyl) methacrylamide)-GFLG-Ama = $Pt(NH_3)_2$, molecular weight 20–25 kDa; Fig. 1] is a novel polymer-conjugated platinum (Pt) compound, designed for tumor targeting. In this copolymer, Pt is attached to a poly-*N*-(2-hydroxypropyl) methacrylamide (pHPMA) backbone via a tetrapeptide spacer (glycine-phenylalanine-leucine-glycine), and an amidomalonic acid chelating agent. Due to the hyperpermeable nature of the neovasculature of tumors, in combination with their limited lymphatic and/or capillary drainage, it is expected that AP 5280 will preferentially accumulate at the tumor site, a phenomenon known as the enhanced permeation and retention effect.[1–4] Subsequently, Pt is released from the polymer intratumorally by lysosomal thiol-dependent proteinases, enzymes known to be elevated in human tumors.[5] Theoretically, AP 5280 administration will thus lead to higher intratumoral Pt concentrations and, therefore, potentially greater efficacy than the currently marketed nonpolymer platinates cisplatin, carboplatin, and oxaliplatin. Preclinical studies show that AP 5280 has a higher therapeutic index than cisplatin and carboplatin when administered to mice implanted with several different types of tumors.[6] On the basis of the promising effects seen in preclinical studies, AP 5280 now is developed further as a potential anticancer agent against solid tumors.

The discovery of a new drug is of practical significance only if the drug can be formulated and presented in a therapeutically acceptable form. The aim of this study was to develop a stable, parenteral dosage form for use in phase I clinical trials. The dosage of AP 5280 is expressed in mg Pt, this being the active component of the compound. The starting dose for phase I clinical studies was set at 90 mg Pt (as AP 5280)/m^2 body surface area (BSA). Initially, a dosage unit content of 200 mg Pt (as AP 5280) was considered most appropriate to cover the expected phase I dosing range, assuming an average BSA of 1.7 m^2 and a maximum dose level of 10 times the starting dose. The development of a suitable parenteral formulation was performed based on the EORTC/CRC/NCI Joint Formulation Working Party guidelines.[7]

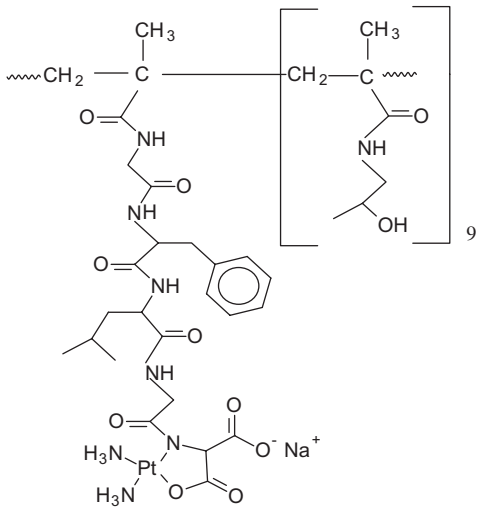

Figure 1. Chemical structure of AP 5280. Molecular weight 24 ± 3 kDa, polydispersity index 1.2–2.3.

MATERIALS AND METHODS

Chemicals and Materials

AP 5280 drug substance (containing 8.0 ± 0.5% Pt) was provided by Access Pharmaceuticals Inc. (Dallas, TX). AP 5280 lyophilized product and phosphate-buffered saline (PBS) were manufactured in-house (Department of Pharmacy and Pharmacology, Slotervaart Hospital, Amsterdam, The Netherlands). Sterile Water for Injections (WfI) (Ecotainer®), 0.9% (w/v) NaCl (normal saline), and 5% (w/v) dextrose were obtained from B. Braun (Melsungen, Germany). Platinum atomic absorption standard, lithium perchlorate, and Hydranal® titrant (2 mg/mL) were purchased from Sigma-Aldrich (St. Louis, MO). Hydrochloric acid 37% (w/v), quinine monohydrochloride dihydrate, and methanol were purchased from Merck (Darmstadt, Germany), Fluka Chimika GmbH (Buch, Switzerland), and Biosolve Ltd. (Amsterdam, The Netherlands), respectively. All chemicals were of analytical grade and used without further purification.

Proton (^{1}H) and Platinum (^{195}Pt) Nuclear Magnetic Resonance Spectroscopy

^{1}H and ^{195}Pt nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DPX 300 spectrometer with a 5 mm multinucleus probe. A variable temperature unit was used to maintain the temperature at 293K for obtaining the ^{1}H NMR spectra and at 298K for the ^{195}Pt NMR spectra. The apparatus was calibrated by using tetramethylsilane at $\delta = 0$ ppm (^{1}H NMR) or K_2PtCl_4 at $\delta = -1614$ ppm (^{195}Pt NMR) as an external reference. The water

signal in the ^{1}H NMR spectra was minimized by using the presaturation technique. Samples (both drug substance and lyophilized product) were prepared by dissolving 80–100 mg in a total volume of 750 μL water with 5% D_2O added for locking the NMR signal.

Infrared Spectroscopy

Infrared (IR) spectra of AP 5280 were recorded on a Model PU 9706 IR spectrophotometer (Philips Nederland B.V., Eindhoven, The Netherlands) by using the potassium bromide pellet technique. The pellet consisted of 2 mg AP 5280 bulk drug or lyophilized product and 300 mg KBr. The ratio recording mode was autosmooth and the scan time 8 min.

Size-Exclusion Chromatography

Chromatographic analyses were performed by using a Spectra System P1000 pump, a Spectra Series AS300 autosampler, and a RI-150 refractive index detector [all Thermo Separation Products (TSP); Fremont, CA]. Separation was achieved using a PL aquagel-OH guard column (7.5 mm ID × 50 mm, particle size 8 μm, Polymer Laboratories, Shropshire, UK), and two PL aquagel-OH MIXED columns (7.5 mm ID × 30 cm, particle size 8 μm; Polymer Laboratories) in series kept at a temperature of 308 K with a Croco-cil® column oven (TSP). The mobile phase consisted of 10 mM $LiClO_4$/methanol 35%/65% (v/v) at a flow of 1.0 mL/min. The injection volume was 100 μL and the run time 30 min. The Size exclusion chromatography (SEC) system was calibrated by using polyethyleneoxide and polyethyleneglycol standards (Polymer Laboratories), with molecular weights ranging from 1080 to 219,300 g/mol.

Samples were prepared by accurately weighing 2–3 mg of AP 5280 drug substance or lyophilized product into an autosampler vial and adding 1.00 mL mobile phase to dissolve the drug substance by gentle swirling.

Flame Atomic Absorption Spectrometry

Total Pt concentrations were measured by using a Perkin Elmer 3100 flame atomic absorption spectrometer (FAAS) Perkin Elmer Inc., Boston, MA. A slit width of 0.7 nm, wavelength of 266 nm, and air-acetylene flame were used. Platinum standards (39.20, 31.36, and 23.52 mg/L) and quality control samples (35.28, 31.36, and 23.52 mg/L) in 0.4 mg/mL pHPMA homopolymer in 0.11 M hydrochloric acid/methanol 50/50% (v/v) were used for quantification of total Pt concentrations. Samples analyzed for their total Pt concentration were diluted with 0.11 M hydrochloric acid/methanol 50/50% (v/v) to yield a theoretical total Pt concentration of approximately 30 mg/L.

Flameless Atomic Absorption Spectrometry

Flameless atomic absorption spectrometry (F-AAS) analysis was performed by using a SpectrA-A 30/40 Zeeman graphite furnace atomic absorption spectrometer (Varian, Techtron Pty Ltd. Victoria, Australia), consisting of a spectrometer, a GTA-75 autosampler, and a DS-15 data station equipped with the Quality Control Protocol software package (Varian). Absorbances were recorded at 265.9 nm, a slit bandwidth of 0.2 nm, and a time constant of 0.05 sec. Argon was used to purge the graphite tube. Platinum concentrations were measured by using a method described previously.[8]

For determination of the content of small Pt species present in AP 5280 drug substance and lyophilized product (“free platinum content”), AP 5280 solutions in WfI were prepared in triplicate at a concentration of 2.0 ± 0.1 mg/mL. For the drug substance, 10 mg AP 5280 was weighed into 5 mL volumetric flasks and dissolved in WfI. The lyophilized product was reconstituted with WfI and further diluted by using WfI. The solutions were stored at ambient temperature (20–25°C) for one hr, after which 2 mL samples were ultrafiltered (45 min, 41 × g, Eppendorf 5403 centrifuge) through a Centricon YM-3 filter (3 kDa cut-off; Millipore, Milford, MA). The Platinum concentration in each ultrafiltrate was analyzed in duplicate.

Release of small Pt species (“liberated platinum content”) was measured in AP 5280 solutions at a concentration of 2.0 ± 0.1 mg/mL in PBS. The solutions were prepared in triplicate as described for the solutions used for determination of the free Pt content. The solutions were stored at 37°C, and, after 3 and 24 hr, 2 mL samples were taken, ultrafiltered through Centricon YM-3 filters, and analyzed. Liberated platinum contents also were

determined in solutions of 125 mg/mL AP 5280 drug substance in normal saline and 5% dextrose. These solutions were stored at room temperature (20–25°C) in the dark, and samples were taken immediately after preparation and after 1, 3, 7, 24, 48, and 96 hr of storage.

Solubility of AP 5280 Drug Substance

The solubility of AP 5280 in water at ambient temperature (20–25°C) was examined by accurately weighing approximately 100 mg AP 5280 in a glass test tube and adding water in 100 μL increments. After each addition, the solution was gently stirred and examined visually under polarized light for complete dissolution of AP 5280 drug substance.

Sterilization

AP 5280 was dissolved in sterile WfI at a concentration of 125 mg/mL. The solution was sterilized by steam sterilization for 15 min at 121°C in a Model 6.6.15 autoclave (Koninklijke Ad Linden B.V., Zwijndrecht, The Netherlands).

Differential Scanning Calorimetry

Transition temperatures and warming characteristics of AP 5280 and pHPMA solutions were examined by differential scanning calorimetry (DSC). These experiments were performed by using a Q1000 V 6.2 DSC in T4 mode equipped with a refrigerated cooling accessory for low temperatures (TA Instruments, New Castle, DE). Samples were placed in an aluminum pan, which was subsequently sealed, and measured against an empty pan as reference. Temperature scale and heat flux were calibrated with indium. Analyses were performed under a nitrogen purge at 50 mL/min. Samples (125 mg/mL AP 5280 or pHPMA homopolymer in water) were cooled to −50°C at a rate of 5°C/min, after which they were heated at a rate of 2°C/min to −2°C. Subsequently, the samples were cooled again to −50°C and heated to 30°C at the same rates. After each cooling and heating step, an isothermal step lasting 5 min was built in. As a reference, water was subjected to the same DSC conditions.

Formulation Process

AP 5280 lyophilized product was prepared aseptically. The formulation solution was prepared to contain 10 mg Pt (as AP 5280)/mL in sterile WfI and dissolved with stirring at ambient temperature, after which it was sterile filtered through a 0.22 μm Millipak 40® filter (Millipore). Subsequently, the formulation solution was filled into washed and sterilized 30-mL type I glass vials (Münnerstädter Glaswarenfabrik GmbH, Münnerstadt, Germany) or 50 mL CZ® resin vials (Daikyo-Seiko, Ltd., Tokyo, Japan). Washed and sterilized siliconized gray bromobutyl rubber stoppers (Type FM 157/1; Helvoet Pharma NV, Alken, Belgium) were positioned on each vial. The vials were loaded into a Model Lyovac GT 4 freeze-dryer (STERIS, Hürth, Germany) at ambient temperature, and lyophilization was performed. After completion of the freeze-drying cycle, the vials were closed pneumatically under vacuum and the vials were retrieved from the freeze-dryer. The product was capped with aluminum caps (Bico Pharma GmbH, Neuss, Germany) and labeled.

In-process controls consisted of integrity testing of the filter; weight variation of the filling volume; determination of the total platinum concentration and bioburden before and after filtration; and monitoring of the product temperature, shelf temperature, chamber pressure, and condensor temperature during freeze-drying. Only clean, sterile, inert materials and glassware were used throughout the manufacturing process. All manipulations took place in a class 100 (A) down-flow cabinet inside a class 100 (B) clean room (Interflow, Wieringerwerf, The Netherlands). Air particle counts in the critical areas and microbiological contamination of the area and the personnel were monitored during the manufacturing process. The manufacture was performed in compliance with the Good Manufacturing Practice guidelines.[9]

Quality Control of AP 5280 Lyophilized Product

AP 5280 lyophilized product was characterized by using the methods also used for the characterization of the drug substance, as well as by visual inspection of the appearance and the color of the pharmaceutical product, and the determination of reconstitution characteristics (rate of dissolution and pH after reconstitution, presence of particles)

and residual moisture content. Furthermore, sterility of the pharmaceutical product was checked by the membrane filtration method according to the United States Pharmacopoeia (USP 24) and the presence of bacterial endotoxins with the limulus amoebocyte lysate test according to the European Pharmacopoeia III.

Residual Moisture Content

Residual moisture levels in AP 5280 lyophilized product were determined by using the Karl Fischer titration method. The content of a vial was quantitatively transferred into the titration unit of a Model 658 KF Titrino apparatus (Metrohm, Herisau, Switzerland) with preconditioned methanol and, subsequently, titrated by using Hydranal® Titrant 2.0 mg H_2O/mL (Sigma-Aldrich). The end-point of the titration was determined biamperometrically.

Stability Studies

Stability of the lyophilized product was examined at the designated long-term storage condition of $5^{\circ}C \pm 3^{\circ}C$ in the dark. After the initial quality control, the samples were tested after 3, 6, and 12 months of storage.

Photostability Studies

Photostability of AP 5280 drug substance and a final product was determined according to (ICH) guideline Q1B.[10] Samples were exposed to light of high intensity emitted by a Suntest CPS$^+$ apparatus with a xenon lamp and an ID-65 window glass filter allowing passage of light of wavelength 320–800 nm, cooled by a Suncool™ chiller (all Atlas Material Testing Technology, Chicago, IL). Samples were exposed to an irradiance level of 68.9 W/m^2 for 24 hr. The following samples were tested: AP 5280 drug substance, spread in a layer of no more than 3-mm thickness in a glass petri dish (Ø 10 cm); AP 5280 lyophilized product in its primary container (both glass vials and CZ resin vials); and AP 5280 lyophilized product pulverized by using a glass rod, taken out of its primary container, and spread in a petri dish as described for the drug substance. Dark controls (prepared exactly as the samples but wrapped in aluminum foil in a manner in which the sample would not come into contact with the aluminum, which is known to interact with platinum) were placed alongside all samples to evaluate the contribution of thermally induced changes. One-centimeter stoppered quartz cells (Hellma GmbH & Co., Müllheim, Germany) filled with a 2% w/v quinine monohydrochloride dihydrate solution (both exposed samples and dark controls) were placed alongside the samples to serve as a chemical actinometric system, ensuring that the specified light exposure was obtained.[10]

RESULTS AND DISCUSSION

Analytical Characterization of AP 5280 Drug Substance

A broad spectrum of analytical techniques is used to fully characterize AP 5280 drug substance. Table 1 shows the methods chosen and results obtained with multiple batches of AP 5280 drug substance. These results were used to define the specifications for the quality control of AP 5280 drug substance. Identification was performed by using ^{1}H NMR and IR spectroscopy, and size exclusion chromatography (SEC) for determination of the molecular weight (MW) characteristics. The size of AP 5280 is an important aspect of its proposed mechanism of action. Furthermore, in order to be efficiently cleared from the body, the MW should be lower than the renal threshold, which, for pHPMA copolymers, was determined to be 45 kDa.[11] Because the way platinum is bound to the chelator determines how easily it is released as a biologically active platinum species, an assessment of the platinum binding was performed by using ^{195}Pt NMR spectroscopy. Flame atomic absorption spectrometry was used to determine the free platinum content and the release profile of liberated platinum of AP 5280 as a measure of the integrity of the molecule, which influences its pharmacokinetics and pharmacodynamics. The assay consisted of total platinum content determination by atomic absorption spectrometry. The combined results of the analyses are able to provide a meaningful picture of AP 5280's structure, size, and integrity.

Preformulation Studies

Due to the pHPMA copolymer, AP 5280 is a highly water-soluble compound. Its solubility is ≥ 500 mg/mL. However, beyond a concentration

Table 1. Overview of the tests performed, results obtained, and specifications set of AP 5280 drug substance (M_w = weight molecular weight, M_n = number molecular weight, PI = polydispersity index).

Test method/item	Results	Specification
1. Appearance	Light brown, flaky substance, free from visible signs of contamination	Light brown, flaky substance, free from visible signs of contamination
2. ^{195}Pt NMR spectroscopy		
A. Identity	A. A single peak at $\delta = -2048 \pm 2$ ppm	A. A single peak at $\delta = -2048 \pm 10$ ppm
B. Purity	B. Absence of other signals than at $\delta = -2048 \pm 2$ ppm	B. Absence of other signals than at $\delta = -2048 \pm 10$ ppm.
3. ^{1}H NMR spectroscopy		
A. Identity	A. Peaks are present at 0.8–1.0, 1.1, 1.6, 1.7–2.0, 3.0–3.3, 3.9, 4.4, 7.3, 7.4, 7.5, and 7.7 ppm.	A. Peaks are present at 0.8–1.0, 1.1, 1.6, 1.7–2.0, 3.0–3.3, 3.9, 4.4, 7.3, 7.4, 7.5, and 7.7 ppm
B. Purity	B. No other accompanying signals are present.	B. No other accompanying signals are present.
4. F-AAS		
A. Free platinum content	A. 2.0 ± 0.1 mg/mL AP 5280 in WfI contains 0.12–0.86% free Pt (with respect to the total Pt content) at room temperature (20–25°C).	A. 2.0 ± 0.1 mg/mL AP 5280 in WfI contains $\leq$1.0% free Pt (with respect to the total Pt content) at room temperature (20–25°C).
B. Liberated platinum content	B. 2.0 ± 0.1 mg/mL AP 5280 in PBS at 37°C releases 0.88 ± 0.18% small Pt species after 3 hr and 2.72 ± 0.85% free Pt after 24 hr (with respect to the total Pt content)	B. 2.0 ± 0.1 mg/mL AP 5280 in PBS at 37°C releases $\leq$ 1.5% small Pt species after 3 hr and $\leq$3.5 % free Pt after 24 hr (with respect to the total Pt content)
5. AAS		
Total platinum content	8.0 ± 0.5% (w/w)	8.0 ± 0.5% (w/w)
6. Size-exclusion chromatography		
A. M_w	A. $M_w = 21.8 - 26.5$ kDa	A. $M_w = 24 \pm 3$ kDa
B. M_n	B. $M_n = 11.8 - 17.0$ kDa	B. $M_n = 14.5 \pm 4$ kDa
C. Polydispersity index	C. PI = 1.5– 1.9	C. PI = 1.2–2.3
D. Shape of the peak	D. Monomodal	D. Monomodal
7. IR spectroscopy	AP 5280 exhibits major absorption bands at approximately 3700–3100 cm^{-1}, 2970 cm^{-1}, 2920 cm^{-1}, 1630 cm^{-1}, 1520 cm^{-1}, 1380 cm^{-1}, 1290–1230 cm^{-1}, 1190 cm^{-1}, 1140–1050 cm^{-1}, and 960–900 cm^{-1}.	AP 5280 exhibits major absorption bands at approximately 3700–3100 cm^{-1}, 2970 cm^{-1}, 2920 cm^{-1}, 1630 cm^{-1}, 1520 cm^{-1}, 1380 cm^{-1}, 1290–1230 cm^{-1}, 1190 cm^{-1}, 1140–1050 cm^{-1}, and 960–900 cm^{-1}.

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of 125 mg/mL, the solution becomes impractically viscous as a consequence of the high polymer concentration. Therefore, the concentration of AP 5280 for the formulation solution during manufacture was set at 125 mg/mL.

The stability of AP 5280 in solution was examined in normal saline and 5% dextrose, both commonly applied infusion solutions, and in sterile WfI. By using F-AAS analysis of ultrafiltered solutions, it was found that during a 4-day storage period at room temperature, the liberated platinum content in the normal saline solution steadily increased to approximately 3% of the total platinum concentration, as is depicted in Fig. 2. Upon dissolution in 5% dextrose, small platinum species were released from AP 5280 during the first 24 hr, after which a plateau level of approximately 1.5% of the total platinum concentration was reached. No significant release of small platinum species was observed for the AP 5280 solution in sterile WfI during 4 days of storage (data not shown). The NMR spectroscopic analysis of this solution after 4 days showed an identical ^{1}H and ^{195}Pt NMR spectrum as immediately after dissolution, confirming that no major structural change had taken place during this time.

Subsequently, stability of the AP 5280 solution in sterile WfI was further examined by exposing the solution to a moist heat sterilization cycle. Although the appearance of the solution did not change upon sterilization, characteristics of the molecule had altered significantly, as is shown in Table 2. Platinum binding had changed, as observed by the shift in the ^{195}Pt NMR resonance from −2046 to −2016 ppm, and other structural changes could be observed in the appearance of two new peaks in the ^{1}H NMR spectrum. Furthermore, both the free and

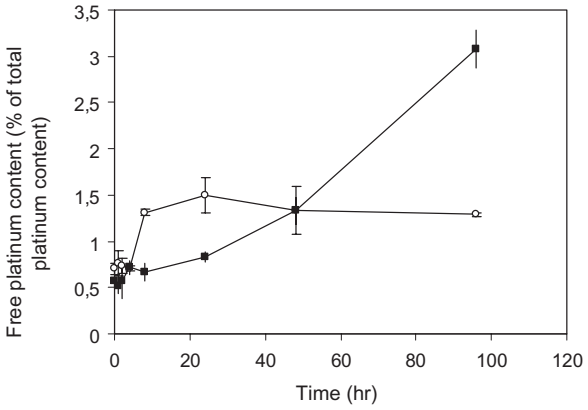

Figure 2. Free platinum content of AP 5280 solutions in normal saline and 5% dextrose (■ = normal saline, ○ = 5% dextrose).

Table 2. Quality control results of AP 5280 before and after sterilization by moist heat (* with reference to the total platinum content, M_w = weight molecular weight, M_n = number molecular weight, PI = polydispersity index).

Test item	Before sterilization by moist heat	After sterilization by moist heat
Appearance	Dark brown solution	Dark brown solution
Foreign insoluble matter	Free from foreign insoluble matter	Free from foreign insoluble matter
^{195}Pt NMR analysis		
1. Identification	1. $\delta = -2046$ ppm	1. $\delta = -2016$ ppm
2. Purity	2. No other signals are present	2. No other signals are present
^{1}H NMR analysis		
1. Identification	1. Peaks are present at 0.8–1.0, 1.1, 1.6, 1.7–2.0, 3.0–3.3, 3.9, 4.4, 7.3, 7.4, 7.5, and 7.7 ppm	1. Peaks are present at 0.8–1.0, 1.1, 1.6, 1.7–2.0, 3.0–3.3, 3.9, 4.4, 7.3, 7.4, 7.5, and 7.7 ppm
2. Purity	2. No other signals are present	2. Extra peaks at 3.7 and 4.0 ppm
F-AAS analysis		
1. Free platinum content*	1. 0.2%	1. 2.2%
2. Liberated platinum content*	2. 3 hr: 0.9% 24 hr: 3.4%	2. 3 hr: 3.2% 24 hr: 5.4%
AAS analysis		
Total platinum content	104.8%	104.8%
SEC		
	$M_w = 26.7$ kDa $M_n = 12.5$ kDa PI = 2.14 Monomodal	$M_w = 16.4$ kDa $M_n = 6.4$ kDa PI = 2.55 Monomodal

liberated platinum content increased. Lastly, analysis by SEC revealed that, although the MW profile remained the same, the weight and number MW decreased substantially, indicative of breakage of the copolymer chains and/or the peptide chains. The polydispersity index increased as compared with the solution before sterilization, providing evidence that a broader range of MWs was present. It could be concluded that sterilization by moist heat of AP 5280 in solution leads to a change in platinum binding, release of small platinum species, and breakage of copolymer and/or peptide chains, and is thus not an option for production of a parenteral formulation.

According to the European Pharmacopoeia III[12] and the ICH Decision Trees for the Selection of Sterilisation Methods,[13] a parenteral product preferentially should be sterilized by terminal sterilization in the primary container. However, this approach clearly caused degradation of AP 5280. Solutions in normal saline and 5% dextrose showed some release of small platinum species and were not considered to be suitable solvents for long-term storage of AP 5280 solutions. Although dissolution of AP 5280 in sterile WfI did not induce changes in AP 5280's structure and integrity for 4 days, the long-term stability of this solution was unlikely, as indicated by stress testing. Therefore, in order to produce a stable product with a sufficiently long shelf life, it was decided to develop a lyophilized dosage form.

Differential Scanning Calorimetry

Figure 3 depicts the DSC thermograms of AP 5280 and pHPMA homopolymer solutions and of water. Two thermal events are observed: the glass transitions (T'_g) with onsets at -14.7°C and -13.2°C (inset Fig. 3) for pHPMA and AP 5280, respectively, and an ice melting endotherm. The glass transition was identified as such because it did not disappear upon repetition of the cooling and heating cycle and was concentration-dependent. The extrapolated onset temperatures of melting of the formulation solution, pHPMA solution, and pure water were -2.0°C, -2.1°C, and -0.9°C, respectively. From the similarities between the thermograms of the pHPMA and formulation solutions, it can be concluded that melting and freezing behavior of AP 5280 in aqueous solution mainly is governed by the pHPMA copolymer, this being the major component of the molecule.

Lyophilization of AP 5280

The product temperature (T_{prod}) as a function of the shelf temperature (T_{shelf}) was determined empirically for the product in the 30-mL glass vials and in the 50-mL CZ resin vials (125 mg/mL AP 5280, 20, and 40 mL fill, respectively) at a chamber pressure (P_c) of 0.15 mbar. Each solution was frozen to -40°C in 3 hr, followed by a freeze-hold lasting 5 hr

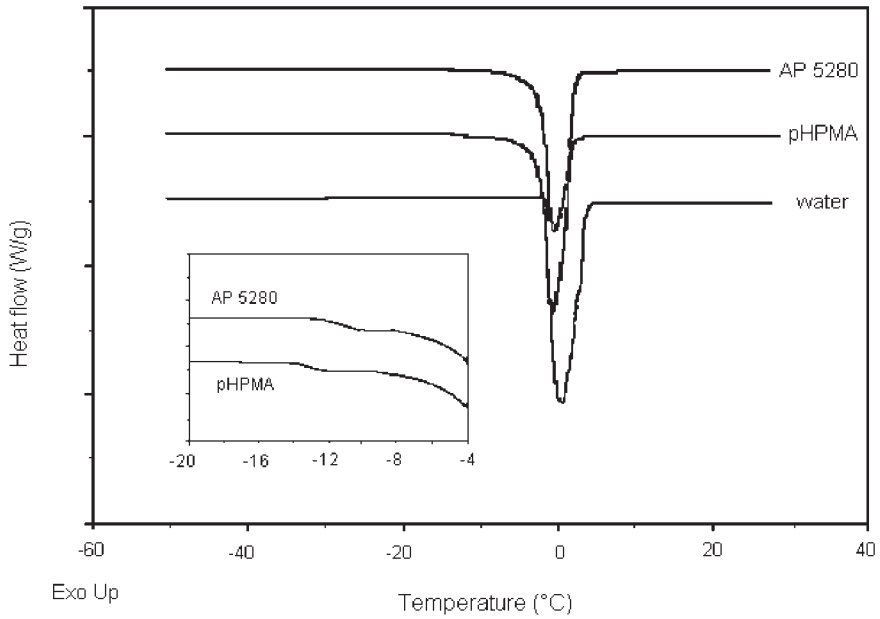

Figure 3. DSC thermograms of AP 5280 (125 mg/mL in water), pHPMA (125 mg/mL in water), and water.

to ensure complete freezing of the vial contents. Subsequently, the P_c was reduced to 0.15 mbar, after which the T_{shelf} was raised in steps of 20°C at a rate of 1°C/min to 0°C, followed by steps of 10°C at the same heating rate to 30°C. After each rise in T_{shelf}, the temperature was kept constant for a period of three hr, after which the T_{prod} was determined. The results of this experiment are shown in Fig. 4. AP 5280 showed a linear increase in T_{prod} with T_{shelf} ($r^2 = 0.99$) that was equal for the glass vials and the CZ resin vials. At a T_{shelf} of +30°C, T_{prod} was found to be −25°C, which was approximately 10°C below the onset of the glass transition temperature and thus considered to be a safe drying temperature.

On the basis of this experiment, lyophilization of the product was performed by using a one-step freeze-drying cycle. Freezing and a freeze-hold remained as described above, after which the P_c was reduced to 0.15 mbar in 1 min and the T_{shelf} raised to +30°C in two hr. The freeze-drying cycle was stopped when T_{prod} reached T_{shelf} (which was approximately 48 hr faster for the 20-mL fill in the glass vials than the 40-mL fill in the CZ resin vials).

Lyophilization of an aqueous AP 5280 solution resulted in a solid, light brown cake with moisture content less than 2% (Table 3). No meltback of ice, and, thereby, collapse of the product, was observed. Quality control of the lyophilized product (Table 3) showed that lyophilization does not affect the structural integrity of AP 5280. Only a slight increase in the free platinum content was observed with respect to AP 5280 drug substance.

Formulation Process Development

Based on the findings in the preformulation studies, an aseptic formulation process, followed by lyophilization, was selected as most appropriate for AP 5280. Freeze-drying eliminates water from the product, thereby reducing chemical degradation rates and increasing the shelf-life.

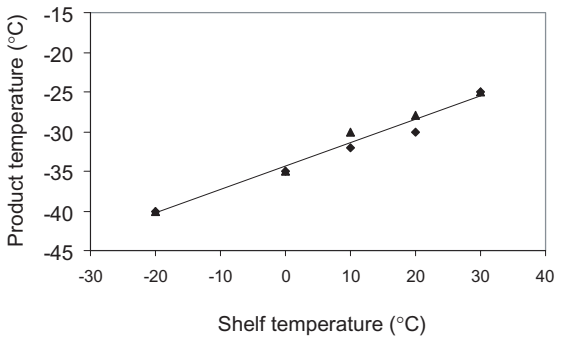

Figure 4. Product temperature as a function of shelf temperature during lyophilization of AP 5280 in glass vials (▲) and CZ-resin vials (◆).

An aqueous solution at a concentration of 10 mg/mL Pt (corresponding to approximately 125 mg/mL AP 5280) was selected as the formulation solution. Preparation of a product for intravenous use preferentially is performed by using as few excipients as necessary, due to possible intrinsic toxicity or activity of the excipient itself or to interactions between the new drug substance and the additives, leading to unknown effects on the activity of the new drug substance.[14] AP 5280 required no bulking agents, excipients to improve solubility, or cryoprotectants in order to manufacture a product of a quality equal to the drug substance. The formulation process thus consisted of dissolution of AP 5280 in sterile WfI. A sterile, hydrophilic 0.22-μm filter for sterile filtration of the formulation solution (Millipak 40, consisting of modified polyvinylidene fluoride membranes in a polycarbonate housing) was found to be compatible with the formulation solution and to comply with pharmacopoeial standards. Peroxide cured silicone tubing (Watson Marlow, Cheltenham, U.K.) was chosen for transport of the formulation solution during the filtration and filling processes because it was inert, autoclavable, and met pharmacopoeial specifications with respect to extractables. The Pt content of the formulation solution before and after filtration was proven to be equal (Pt content before filtration: 8.4% ± 0.2%, Pt content after filtration: 8.4% ± 0.3%, $n = 3$), thus no retention of AP 5280 by filter tubing used in the manufacturing process took place.

One major problem was encountered upon manufacture of AP 5280 lyophilized product. Initially, the platinum content was set at 200 mg Pt per vial. The batches produced by filling 20-mL formulation solution into 30-mL glass vials showed a breakage rate ranging from 11% to 33%. Breakage of vials has been described previously for solutions containing mannitol and is usually the result of large fill heights and volumes, high concentrations of the substance to be lyophilized, and/or high freezing rates.[15,16] The concentration of the AP 5280 formulation solution could not be decreased, because the dose per vial had to remain 200 mg Pt/vial or even increase as the clinical study proceeded. For mannitol solutions, it was found that a freezing rate between 0.1 and 1.0°C/min decreased vial breakage as compared with faster and slower freezing rates.[15] As the freezing rate of AP 5280 formulation solution

Table 3. Specifications for AP 5280 lyophilized product and stability of AP 5280 lyophilized product in glass and Daikyo CZ-resin vials (* with reference to the total platinum content).

		Glass vials (200 mg Pt/vial)		CZ-resin vials (400 mg Pt/vial)	
Test item	Specification	t_0	12 months at 2–8°C	t_0	6 months at 2–8°C
Appearance	Light brown, lyophilized cake	Conforms	Conforms	Conforms	Conforms
Reconstitution					
1. Rate	1. Complete reconstitution (5% dextrose) ≤ 1 hr	1. Conforms	1. Conforms	1. Conforms	1. Conforms
2. Foreign insoluble matter	2. A clear, dark brown solution, free from foreign insoluble matter	2. Conforms	2. Conforms	2. Conforms	2. Conforms
^{195}Pt NMR analysis					
1. Identification	1. A single peak at $\delta = -2048 \pm 10$ ppm	1. $\delta = -2047$ ppm	1. $\delta = -2046$ ppm	1. $\delta = -2048$ ppm	1. $\delta = -2047$ ppm
2. Purity	2. Absence of other signals than at $\delta = -2048 \pm 10$ ppm	2. Conforms	2. Conforms	2. Conforms	2. Conforms
^{1}H NMR analysis					
1. Identification	1. ^{1}H NMR spectrum reference standard = ^{1}H NMR spectrum final product. Peaks at 0.8–1.0, 1.1, 1.6, 1.7–2.0, 3.0–3.3, 3.9, 4.4, 7.3, 7.4, 7.5, and 7.7 ppm	1. Conforms	1. Conforms	1. Conforms	1. Conforms
2. Purity	2. No other accompanying signals present	2. Conforms	2. Conforms	2. Conforms	2. Conforms

F-AAS analysis					
1. Free platinum content*	1. ≤ 1.0% free Pt (with respect to total Pt content) in water	1. 0.72%	1. 0.42%	1. 0.79%	1. 0.67%
2. Liberated platinum content*	2. ≤ 2.0% free Pt after 3 hr and ≤ 5.0% free Pt after 24 hr (with respect to total Pt content) in PBS at 37°C	2. $t = 3$: 0.86% $t = 24$: 2.11%	2. $t = 3$: 1.15% $t = 24$: 2.72%	2. $t = 3$: 1.09% $t = 24$: 3.48%	2. $t = 3$: 0.79% $t = 24$: 2.23%
AAS analysis					
1. Total platinum content	1. 90.0–110.0% of the labeled Pt content	1. 107.6%	1. 108.6%	1. 110.1%	1. 99.8%
2. Content uniformity	2. Contents of 10 vials are within 90.0–110.0% of the labeled Pt content with a relative standard deviation ≤ 6.0%	2. Conforms RSD = 1.1%	2. —	2. Conforms RSD = 3.6%	2. —
SEC	All SEC parameters (M_w, M_n, PI, and shape of peak) within 90.0–110.0% of the AP 5280 drug substance used for the manufacture of the final product	Conforms	Conforms	Conforms	Conforms
pH after reconstitution	6.2–7.2	6.7	6.7	6.6	6.6
Bacterial endotoxins	< 10 IU /vial	Conforms	Conforms	Conforms	Conforms
Sterility	Sterile	Conforms	Conforms	Conforms	Conforms
Residual moisture content	< 3.0% (w/w)	0.99%	1.08%	0.71%	1.45%

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was already 0.3°C/min, alteration of this rate was not expected to reduce breakage. Therefore, the use of larger vials, and, thereby, reduction of the fill height, was investigated. When 50-mL glass vials were used, no vial breakage was observed. Therefore, breakage of vials was apparently a result of the large fill height.

As the phase I clinical trials progressed and the administered doses of AP 5280 increased, it was decided to upscale the manufacturing process by doubling the content of AP 5280 per vial, as this would be more convenient for preparation of the infusion solutions. However, if the fill height in the 50-mL glass vials was increased, similar breakage as experienced for the 20-mL fill volume in the 30-mL glass vials could be expected. Therefore, the use of CZ resin vials was considered, as these are claimed to be unbreakable. Preparation of a test batch showed that the quality of the lyophilized product was not influenced by the change in primary container (Table 3). As transport of CZ resin vials is more convenient than transport of glass vials due to their smaller likelihood of breakage and lower weight (and thus reduction of transport costs), the switch to CZ resin vials was made. Although the CZ resin vials may show ingress of oxygen and may thus be inappropriate for packaging oxygen-sensitive formulations,[17] AP 5280 has not shown any sign of oxygen sensitivity. Furthermore, stability studies were performed to monitor the quality of AP 5280 lyophilized product in time and determine if it remained unchanged upon storage.

Stability Studies

Storage of the Lyophilized Product

Table 3 shows the results obtained thus far for the stability studies. To date, AP 5280 lyophilized product in glass and in CZ resin vials proved to be stable for 12 and 6 months, respectively, when stored at 2–8°C. No major changes were observed in any of the parameters, although the CZ resin vials show a slight increase in moisture content, but the results still conform to specifications. Therefore, refrigerated conditions (2–8°C) were chosen as the storage condition of AP 5280 lyophilized product.

Photostability

AP 5280 drug substance and AP 5280 lyophilized product inside and outside its glass and its CZ resin vials underwent photostability studies. The average temperature during exposure was 28°C.

The results obtained for the photostability study are shown in Table 4. Although the free Pt content of the exposed sample of AP 5280 drug substance had quadrupled as compared with the dark control, the results still conformed to specifications. However, liberated Pt content after 24 hr, the weight molecular weight, and the polydispersity index of the exposed sample were out of specification. The results obtained for AP 5280 drug substance after exposure to light indicated that it is photolabile. Therefore, AP 5280 drug substance should be stored protected from light.

For AP 5280 lyophilized product, the product exposed in the glass vials shows a pH after reconstitution that is out of specification and the product taken out of its glass primary container exposed in a petri dish does not conform to the SEC specifications. The weight molecular weight of this product exceeded the upper limit set for this parameter. The SEC results indicate that the lyophilized product, when removed from the glass primary container, experienced further polymerization upon exposure to light of high intensity, just like the drug substance. The product inside the glass primary container showed no such changes in molecular weight parameters and it, thus, seems that the glass vial provided protection against this effect. The product in the glass vials exposed to light of high intensity (both inside and out of its glass primary container) showed an increase in the liberated and free Pt content when compared with the dark controls, but the results still comply with the specifications.

For the product in the CZ resin vials, both exposed samples (inside and out of the primary container) showed a liberated Pt content after 3 hr that was out of specification. Furthermore, the exposed sample in the primary container also had a free Pt content that deviated significantly from the dark control. This indicates that AP 5280 lyophilized product inside and out of the CZ resin vials is photolabile.

In conclusion, AP 5280 lyophilized product, when produced in glass or in CZ resin vials, both inside and out of the primary container, is sensitive to photodegradation and should be stored in the dark.

Stability After Reconstitution and Dilution to Infusion Concentrations

Stability of AP 5280 after reconstitution and dilution to infusion concentrations has been

Table 4. Photostability of AP 5280 drug substance and lyophilized product (inside and out of the primary container).

	Drug substance		Glass vials				CZ-resin vials			
Test item	Dark control	Exposed	Dark control in primary container	Exposed in primary container	Dark control in petri dish	Exposed in petri dish	Dark control in primary container	Exposed in primary container	Dark control in petri dish	Exposed in petri dish
Appearance	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Reconstitution	—	—								
1. Rate			Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	1. Does not conform
2. Foreign insoluble matter			Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	1. Conforms	2. Conforms
^{195}Pt NMR analysis										
1. Identification	1. $\delta =$ −2045 ppm	1. $\delta =$ −2051 ppm	1. $\delta =$ −2052 ppm	1. $\delta =$ −2049 ppm	1. $\delta =$ −2046 ppm	1. $\delta =$ −2048 ppm	1. $\delta =$ −2049 ppm	1. $\delta =$ −2047 ppm	1. $\delta =$ −2050 ppm	1. $\delta =$ −2049 ppm
2. Purity	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms
^{1}H NMR analysis										
1. Identification	1. Conforms	1. Conforms	1. Conforms	1. Conforms	1. Conforms	1. Conforms	1. Conforms	1. Conforms	1. Conforms	1. Conforms
2. Purity	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms	2. Conforms
F-AAS analysis										
1. Free Pt content	1. 0.12%	1. 0.48%	1. 0.20%	1. 0.21%	1. 0.10%	1. 0.29%	1. 0.85%	1. 1.38%	1. 0.97%	1. 1.07%
2. Liberated Pt content	2. 3 hr: 0.58% 24 hr: 2.06%	2. 3 hr: 1.30% 24 hr: 3.66%	2. 3 hr: 0.55% 24 hr: 2.30%	2. 3 hr: 0.74% 24 hr: 2.70%	2. 3 hr: 0.53% 24 hr: 2.29%	2. 3 hr: 0.86% 24 hr: 2.84%	2. 3 hr: 0.92% 24 hr: 2.57%	2. 3 hr: 1.85% 24 hr: 3.27%	2. 3 hr: 0.85% 24 hr: 2.38%	2. 3 hr: 1.90% 24 hr: 3.29%
AAS analysis										
Total Pt content	8.21%	8.34%	102.3%	102.3%	99.1%	100.2%	100.3%	102.6%	96.9%	91.8%
SEC										
	$M_w = 24.3$ kDa $M_n = 10.8$ kDa PI = 2.26 Monomodal	$M_w = 33.5$ kDa $M_n = 11.3$ kDa PI = 2.99 Monomodal	$M_w = 21.7$ kDa $M_n = 11.5$ kDa PI = 1.89 Monomodal	$M_w = 22.1$ kDa $M_n = 10.6$ kDa PI = 2.08 Monomodal	$M_w = 23.7$ kDa $M_n = 10.8$ kDa PI = 2.18 Monomodal	$M_w = 28.5$ kDa $M_n = 10.7$ kDa PI = 2.65 Monomodal	$M_w = 24.7$ kDa $M_n = 11.3$ kDa PI = 2.20 Monomodal	$M_w = 25.2$ kDa $M_n = 10.5$ kDa PI = 2.40 Monomodal	$M_w = 24.6$ kDa $M_n = 10.9$ kDa PI = 2.25 Monomodal	$M_w = 26.6$ kDa $M_n = 11.0$ kDa PI = 2.40 Monomodal
pH after reconstitution	—	—	7.0	7.3	—	—	6.9	7.0	—	—

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described elsewhere.[18] AP 5280 should be reconstituted and diluted to an infusion concentration with 5% dextrose and administered within 8 hr after preparation to ensure that less than 1.0% of the total Pt concentration is present as free Pt.

CONCLUSIONS

Manufacture of a lyophilized product of the investigational polymer-conjugated anticancer agent AP 5280 from an aqueous solution has provided the formulation method with the least impact on the integrity of AP 5280. Due to breakage of glass vials and in the course of upscaling CZ resin vials were chosen as the primary container. AP 5280 lyophilized product in CZ resin vials is stable for six months when stored at refrigerated conditions and needs to be stored protected from light. AP 5280 is currently in phase I clinical trials. The first clinical data[19] indicate that the proposed formulation is fully applicable in the clinical setting.

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