Physicochemical Characterization, Solubilization, and Stabilization of 9-Nitrocamptothecin Using Pluronic Block Copolymers

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ABSTRACT: Solid-state properties and physicochemical characteristics of 9-nitrocamptothecin (9NC) were investigated with a view of molecular and bulk level understanding of its poor aqueous solubility and hydrolytic instability that prevent efficient drug delivery and pharmacological activity. 9NC bulk drug substance was found to be a nonhygroscopic, yellowish crystalline solid with long rectangular prism-shaped particle morphology and a sharp melting point at 264°C. Hydrolysis of 9NC-lactone occurs above pH 4, whereas complete conversion of lactone to carboxylate was recorded above pH 8. At saturated conditions, appreciable concentrations of 9NC-lactone were detected at pH as high as 11. 9NC undergoes oxidation in the presence of dimethyl sulfoxide with formation of 9NC-N-oxide. The total solubility of lactone and carboxylate forms of 9NC in deionized water was found to be less than 5 μg/mL, whereas the solubility of 9NC-lactone in aqueous acidic media was determined to be approximately 2.5 μg/mL. Incorporation of 10% pluronic copolymers P123, F127, and F68 in 10 mM HCl increased 9NC solubility by 13-fold, eightfold, and fivefold, respectively. The thermodynamic stability of drug-loaded pluronic micelles was evaluated under isothermal variable volume conditions and found F127, among all poloxamers, to offer the best hydrolytic protection efficacy for 9NC. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3653–3665, 2013

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INTRODUCTION

Camptothecin (CPT) and many of its different analogues are potent cytotoxic pentacyclic plant alkaloids first isolated from the Asian tree *Camptotheca acuminata*, and have shown a unique mechanism of anticancer action against a broad range of tumors by specifically inhibiting the nuclear enzyme topoisomerase I.[1–11] The remarkable success of CPT against various human tumors in preclinical studies prompted clinical investigations. However, the therapeutic application of unmodified CPT was hindered because of its poor water solubility. The initial approach taken to use water-soluble sodium salt form of CPT for cancer treatment produced severe toxicity.[12,13] Subsequent intensive research and improved understanding of the structure activity relationship led to the synthesis of several CPT derivatives that were more suitable for clinical investigations. Advances in chemical structural modification of the CPT molecule led to the synthesis of more efficient water-soluble and water-insoluble classes of CPTs. Two compounds in this class, topotecan (Hycamtin®) and irinotecan (Camptosar®), have been approved by the US FDA, and are currently being used in the clinical treatment of ovarian and colon cancer.[14–17] 9-nitrocamptothecin (9NC), an orally administered poorly water soluble semisynthetic new analog of CPT exhibited a very promising preclinical *in vitro* and *in vivo* antitumor activity and underwent phase III trials for the treatment of newly diagnosed and refractory pancreatic cancer.[18–24]

Similar to other CPT analogues, 9NC has a pentacyclic ring structure with quinoline (rings A and B), indolizinone (rings C and D), and an α-hydroxy δ-lactone moiety (ring E).[25] The intact lactone ring is an important structural requirement for successful interaction with the topoisomerase I and for passive diffusion of drug into cancer cells.[26–29] As with all CPT analogues, the α-hydroxy δ-lactone moiety of 9NC undergoes a pH-dependent nonenzymatic reversible hydrolysis above pH 5, to a ring-opened carboxylate form with loss of pharmacologic activity.[30,31] Moreover, association of CPT lactone and carboxylate forms with human serum albumin further affects their *in vivo* equilibrium and consequently their pharmacological activity.[32–34] The instability along with its poor water solubility leads to a very challenging path for developing efficient drug delivery systems.

Theoretical understanding of micelle formation with regard to prediction of size and size distribution of micelles formed by many amphiphiles have been greatly improved.[35] In recent years, the potential of polymeric micelles to solubilize poorly water-soluble drugs has been well reported.[36–44] Furthermore, the core–shell micellar structure that provides an adequate protection of the liable lactone ring of CPT molecule in physiological conditions has been a major focus.[45,46] In this work, we have investigated various critical physicochemical parameters of 9NC and explored a micellar formulation approach toward improving its aqueous solubility and hydrolytic instability in physiological conditions. Quite frequently, preparation of drug-loaded micelles involves the use of organic solvent. In this study, aqueous acidic solution has been used to prepare 9NC-drug-loaded micelles with different commercially available pluronic

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block copolymers to overcome the undesirable effect of organic solvent. Additionally, a real-time nonperturbing first derivative spectroscopy technique[47–52] has been utilized for the simultaneous determination of non-equilibrium concentrations of 9NC-loaded micellar solutions. It was found that all poloxamers enhanced the solubility and stability of 9NC in micellar formulations, but F127 exhibited the best hydrolytic protection.

MATERIALS AND METHODS

The 9NC, (4-ethyl-4-hydroxy-9-nitro-1H-Pyrano[3′,4′:6,7] indolizino[1,2-b]quinoline-3,14(4H,12H)-dione, CAS Number: 86639-62-5, $C_{20}H_{15}N_3O_6$, 393.349 g/mol, mass fraction purity $\geq$ 0.99 was purchased from Afine Chemicals (Hangzhou, Zhejiang, China). Elemental analysis of this compound conducted by Robertson Microlit Laboratories (Madison, New Jersey) yielded mass purity of at least 0.99 (Calculated: C, 61.07; N, 10.69; H, 3.82. Found: C, 60.84; N, 10.64; H, 3.69). All experiments were performed using analytical grade reagents. Deionized water was obtained from a Barnstead NANOpure water system (Barnstead, Dubuque, Iowa). Dimethyl sulfoxide (DMSO; HPLC grade, 99%), 1-octanol (HPLC grade 99%), and all other analytical grade reagents used were purchased from Sigma–Aldrich (St. Louis, Missouri). Pluronic block copolymers P123, F68, and F127 of different average molecular weights of 5750, 8400, and 12,600 g/mol, respectively, were obtained from BASF Corporation (Florham Park, New Jersey).

SOLID-STATE CHARACTERIZATION

Scanning Electron Microscopy

Particle shape and morphology of the 9NC powder were investigated by scanning electron microscope JSM-5610LV (JEOL USA, Inc., Peabody, Massachusetts), operating between 0.5 and 5.0 kV. Samples were prepared by mounting a piece of double-sided adhesive carbon tape on an aluminum stub and then placing a portion of a sample on the tape. The sample was sputter coated with gold to be made electrically conductive and then imaged.

Particle Size Measurement of Dry Powder

Particle size of 9NC drug powder was determined using laser diffraction particle sizer (Mastersizer 2000; Malvern Instruments Inc., Westborough, Massachusetts) following a wet dispersion method. Deionized water was used as dispersion medium. 9NC powder was added into the dispersion unit of the machine and stirred well with an inbuilt propeller type blade at a rate of 2500 rpm. Measurements were made after 1 min of stirring setup as the equilibration time.

X-Ray Powder Diffraction

X-ray powder diffraction (XRPD) of 9NC was performed using a Philips X'Pert System (Philips Analytical, Westborough, Massachusetts) with a CuK radiation source ($\lambda = 1.54$ Å). Samples were prepared for analysis by compressing 9NC powder into an aluminum sample holder (8 mm × 11 mm × 1 mm) using a glass slide. The sample holder was then placed inside the system and scanned from 5° to 45° 2θ using a scan rate of 1° 2θ/min at room temperature. The current and voltage were set at 30.0 mA and 40 kV, respectively. Equipment performance was tested using a National Institute of Standards and Technology traceable Mica standard for peak position.

Differential Scanning Calorimetry

Differential scanning calorimetric (DSC) study was performed on 9NC using a Tzero™ Q1000 (TA Instruments, New Castle, Delaware) equipment, at a heating rate of 10°C/min under nitrogen purge. The calorimeter (TzeroTM Q1000; TA Instruments) was first calibrated using pure indium (melting point 156.6°C). The sample was accurately weighed in the range of 2–6 mg into aluminum pan followed by crimping with aluminum lid. Each experiment cycle was conducted by conventional DSC heating ramp starting from 25°C to 350°C at 10°C/min. Data were analyzed using the Universal Analysis™ 2000 software (TA Instruments).

Melting Point by Capillary Apparatus

The melting point of 9NC was determined by a Thomas Hoover Uni-melt Capillary Melting Point Apparatus (Thomas Scientific, Swedesboro, New Jersey). Capillary tubes were filled with the dry powder and placed in the melting point apparatus for melting point determination. The samples were heated electrically via an oil bath and the melting process was observed through magnifying lens.

Thermogravimetric Analysis

The effect of temperature on gravimetric changes of 9NC was characterized using a TGA Q500 Thermogravimetric Analyzer from TA Instruments. Samples were in the range of 1–15 mg. Each cycle was conducted by conventional thermogravimetric analysis (TGA) heating ramp starting from 20°C to 350°C at a linear scanning rate of 10°C/min. Universal Analysis™ 2000 software from TA Instruments was used for data analysis. The equipment performance was verified from the Curie temperature of Nickel and by confirming a 2% weight loss of ethoxyethylacetate in polyol.

Moisture Adsorption/Desorption Measurement

Moisture adsorption/desorption isotherms of 9NC powder were studied by using the Symmetric Vapor Sorption SGA-100 (VTI Corporation, Inc., TA Instruments, New Castle, Delaware). Approximately 3–5 mg sample was placed in the sample pan and dried at 60°C for 1 h and sorption–desorption cycle was conducted at 25°C at relative humidity range from 10% to 90%. Changes in sample weight were recorded using an electronic microbalance. Calibration of the instrument was conducted with an appropriate weight placed on the sample pan.

HPLC Instrumentation

Chromatographic analysis of 9NC-lactone and 9NC-carboxylate was carried out in a reverse-phase column of C_{18}, 150 mm × 4.60 mm, 5 μm particle size (Phenomenex Gemini, Torrance, California) with a Waters HPLC system (Waters Corporation, Milford, Massachusetts) composed of a 717 plus autosampler, a single 515 pump, and an UV absorbance detector. The mobile phase was a mixture of acetonitrile and sodium dihydrogen phosphate buffer (0.1 mM adjusting the pH to 5.5 with acetic acid) in a ratio of 30:70. The flow rate was 1 mL/min and the wavelength was 370 nm. Calibration curves for the 9NC-lactone and 9NC-carboxylate species were also constructed and were linear over the range