he first enzyme-catalyzed therapeutic activation compound to enter clinical studies—NB1011—is a developmental candidate for fluoropyrimidine-resistant metastatic or relapsed colorectal cancers. NB1011 is a nucleotide phosphoramidate, (E)-5-(2-bromovinyl)-2'-deoxy-5'-uridyl phenyl L-alaninylphosphoramidate. The compound targets tumor cells with high levels of thymidylate synthase (TS) expression. Cytotoxic species are generated following processing of the intracellular form of NB1011 by TS. TS is overexpressed in several cancers, especially those treated with 5-fluorouracil (5 FU), thus leading to drug resistance and treatment failure.

NB1011 is a white to off-white powder that demonstrates limited solubility in aqueous solution. Although NB1011 is soluble in cosolvents, it precipitates upon dilution in saline. The work described in this article was directed toward the development of a suitable parenteral formulation for toxicological and clinical evaluation. The objective was to develop a nonaqueous small-volume parenteral (SVP) formulation suitable for intravenous infusion upon dilution. The concentration of the diluted formulation should be at least 5–8 mg/mL. The formulation must be easy to manufacture and be shelf stable.

**Materials and methods**

**Materials.** NB1011 was received from NewBiotics Inc. (San Diego, CA). All other ingredients were acquired from either Sigma (St. Louis, MO) or Mallinkrodt (Phillipsburg, NJ).

**HPLC method.** A validated high-performance liquid chromatography (HPLC) method was used for analyzing NB1011 samples. The reverse phase method used for quantitating the drug substance involved a Phenomenex (T orrance, CA) Luna C18 column (150 × 4.6 mm, 100 Å), the mobile phase used to elute the compound consisted of 60:40 methanol:0.01 M potassium phosphate buffer v/v at a flow rate of 1 mL/min, and the eluent was monitored at 293 nm.

**Thermal gravimetric analysis (TGA).** Thermal behavior was analyzed on a TA Instruments (New Castle, DE) 2050 thermal gravimetric analyzer with an argon purge and a heating rate of 5 °C/min.

**Differential scanning calorimetry (DSC).** All scans were run on a TA Instruments 2920 differential scanning calorimeter. Samples were prepared in sealed aluminum pans and heated at a rate of 10 °C/min from −40 to +200 °C in an argon atmosphere.
The drug has higher solubility at alkaline pH conditions. However, pH stability studies performed at 50 °C indicated that it might also be prone to degradation at alkaline pH. The drug undergoes a pseudo-first-order degradation catalyzed by acid and base; the degradation was much more rapid under alkaline conditions (see Figure 1). Although the drug exhibited higher solubility at pH >7.0, the base-catalyzed degradation prohibited the development of a shelf-stable formulation. In addition, preformulation data indicated that an aqueous formulation for parenteral administration could not be developed. Hence, three formulation approaches were evaluated to develop a small-volume parenteral: the use of cosolvent systems, complexation with cyclodextrins, and micellar solubilization.

**Cosolvent systems**
Cosolvents can alter the solubility and stability of compounds (2). These two parameters can be exploited during formulation to produce a commercially acceptable and elegant parenteral product. Cosolvents facilitate the development of a concentrated formulation that allows production of a dosage form to be filled in ampuls or vials. The contents of the ampul or vial is then diluted before the drug is administered. The important factor in this approach is to develop a formulation that upon dilution will have enough cosolvents to be above the equilibrium solubility to avoid precipitation of drug in the infusion bag or upon injection (3).

The approach was evaluated using pharmaceutically accepted solvents (see Table I). Unfortunately, all of these formulations precipitated upon dilution with 0.9% sodium chloride in water. Hence, complexation with cyclodextrins was investigated next.

**Complexation with hydroxypropyl beta cyclodextrin (HPβCD)**
Complexation is an equilibrium process. Most drugs form a 1:1 complex with cyclodextrins (4). The literature contains numerous examples in which complexation with cyclodextrins drastically improves the solubility and stability of the drugs (5). HPβCD endotoxin-controlled has been used for parenteral applications.

HPβCD can complex NB1011 and improve its solubility (see Figure 2). The solubility increases linearly with an increase in cyclodextrin concentration, thereby suggesting that the com-
NB1011 is soluble in hydroalcoholic solutions of HPβCD, which matches well with the experimentally determined intrinsic solubility of NB1011 in water.

The maximum solubility obtained with 40% HPβCD was 14.6 mg/mL. The amount of HPβCD could not be increased further because of the dramatic increase in viscosity of the formulation, which made it impractical for manufacturing a small-volume parenteral. Researchers replaced portions of aqueous phase with ethanol to minimize the increase in viscosity and enhance solubility. NB1011 is soluble in a hydroalcoholic solution of HPβCD (see Table II). The solubility increases dramatically, but all of the hydroalcoholic cyclodextrin solutions precipitated NB1011 upon dilution with 0.9% saline.

### Micellar solubilization

Solubilization of water-insoluble drugs by surfactant micelles was investigated (6). The surfactant micelle not only enhances the solubility but also improves the stability of labile drugs. Selection of the surfactants for the current investigation was based on each surfactant’s toxicological profile and its history of use in marketed products. Three surfactants—

- d-α tocopheryl polyethylene glycol 1000 succinate (TPGS, Eastman Chemical Co., Kingsport, TN),
- Solutol (BASE, Shreveport, LA), and
- Polysorbate80 (BASF)—were selected for use in formulation development.

NB1011 is soluble in various combinations of surfactant systems (see Table III). As expected, the solubility of NB1011 increased with an increase in the amount of surfactant. In the case of TPGS, the viscosity of the formulation that contained >20% TPGS increased dramatically because the micelles of TPGS reorient to form the cubic-phase gel.

The solubility of NB1011 was further evaluated in nonaqueous formulations that contained the surfactants. The nonaqueous formulations were developed on the basis of polyethylene glycol-300, propylene glycol, and ethanol. The solubility of NB1011 was much higher in these formulations, and all of them prevented precipitation of the drug upon dilution with saline.

The formulation containing polyethylene glycol-300/ethanol/Polysorbate80 (50:40:10 w/w/w/) showed the solubility of NB1011 to be ~50 mg/mL. On the basis of the toxicological profile of the excipients, this formulation was selected for further development. The formulation was evaluated to study the stability under accelerated conditions and the effect of autoclaving.

When the formulation was exposed to two autoclave cycles, NB1011 showed no evidence of degradation. Also, an informal stability study at 50 °C for four weeks showed minimal degradation. The observed rate constant for the pseudo–first-order degradation was 0.0026/day, which strongly suggested that the formulation would show adequate shelf stability at room temperature.

### Conclusion

A study to develop a shelf-stable formulation of a cancer treatment evaluated three approaches: the use of cosolvent systems, complexation with cyclodextrins, and micellar solubilization. Micellar solubilization with Polysorbate80, along with cosurfactants, was successful for developing a nonaqueous small-volume parenteral formulation. The formulation proved suitable for intravenous administration because it showed no evidence of precipitation upon reconstitution in a solution of 0.9% sodium chloride and because of the favorable toxicological profile of the excipients. Finally, the formulation was easy to manufacture and showed adequate shelf stability at room temperature.

### References