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# The removal of Cremophor® EL from paclitaxel for quantitative analysis by HPLC–UV

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**Abstract**

A novel method for analysis of hydrophobic drug molecules in matrices that contain Cremophor® EL (CrEL) is presented. The method utilized a precipitation technique involving mercuric chloride in a reaction with CrEL to form an insoluble complex in an ethanol matrix. The hydrophobic drug molecule was then analyzed by HPLC–UV without interference from CrEL. Nuclear magnetic resonance and infrared spectroscopy indicated that the mechanism of precipitation involves the reaction of mercuric chloride with the ether bond of CrEL. Analysis by HPLC with UV detection of paclitaxel and related substances was used to verify that the reaction is specific toward CrEL.


*Keywords:* Analysis of paclitaxel; Removal of Cremophor® EL; LC–UV

## 1. Introduction

Cremophor® EL (CrEL) is a solubilizer manufactured by BASF and used in a number of finished drug product formulations. These formulations are typically injectable products where the active pharmaceutical ingredient (API) is not readily soluble in aqueous or aqueous/ethanol solutions. In particular, CrEL solubilizes hydrophobic drugs by formation of a micelle, which creates a hydrophobic environment for the API. Among the more popular injectable drugs which incorporate CrEL are Taxol®, Vumon®, Vinpocetine®, and Sandimmune®. CrEL is a viscous liquid that is formed by the reaction of ethylene oxide with castor oil at a molar ratio of 35:1 [1]. A major component (80%) of CrEL consists of a hydrophobic glycerol–polyethylene glycol ricinoleate bonded to the hydrophilic polyethylene glycols and ethoxylated glycerol [1].

While CrEL does an excellent job solubilizing the drug components, it makes the analytical task of quantification of the drug substance including impurities difficult. Due to the purity of the raw materials used in CrEL, the compound itself is actually a distribution of similar compounds with varying molecular weights. The complete composition of CrEL has been studied, but is not well characterized [2]. Because of the typical concentration used and the UV absorbance, CrEL causes multiple interfering peaks when HPLC analysis with UV detection is used. Since HPLC–UV is the overwhelming choice for stability-indicating methods for the analysis of potency and related substances, the presence of CrEL makes such quantitation difficult.

Previous work has been successful at separating paclitaxel and related substances using HPLC [3,4] and MEKC [5]. These methods generally take advantage of the greater retention of CrEL relative to paclitaxel to achieve resolution. In addition to reduced sensitivity, CrEL interferes with the resolution of derivatized paclitaxel as well as late eluting impurities. Increased sensitivity was obtained when solid-phase extraction was employed to remove CrEL for the analysis of paclitaxel and metabolites [6]. However, as is often the case with SPE treatment, a loss of paclitaxel occurred during the SPE preparation.

A sample preparation technique was developed to remove the CrEL from the finished product sample in order to analyze for potency and related substances. The CrEL was removed via a reaction with mercuric chloride to form an insoluble complex. HPLC analysis using UV detection was then performed to verify the specificity of the reaction. Nuclear mag-

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netic resonance and infrared spectroscopic techniques were used to elucidate the reaction mechanism so that a prediction could be made for the utilization of the technique with other compounds.

The choice of paclitaxel as API for analysis after precipitation of CrEL was made for several reasons. Firstly, paclitaxel has a number of well-characterized related substances that are readily available for use in developing methodology. Secondly, paclitaxel is a highly prescribed oncology drug. Finally, the pharmaceutical industry is currently researching multiple derivatives of paclitaxel.

## 2. Experimental

### 2.1. Chemicals and reagents

The following chemicals were investigated as potential reactants with which to form an insoluble complex with CrEL: mercuric chloride (Sigma–Aldrich, St. Louis, MO), mercuric iodide (Sigma–Aldrich, St. Louis, MO), mercuric bromide (Sigma–Aldrich, St. Louis, MO), barium chloride (Sigma–Aldrich, St. Louis, MO), mercuric acetate (Sigma–Aldrich, St. Louis, MO), resorcinol (Sigma–Aldrich, St. Louis, MO), cobalt chloride (Sigma–Aldrich, St. Louis, MO), zinc chloride (Sigma–Aldrich, St. Louis, MO), nickel chloride (Sigma–Aldrich, St. Louis, MO), silver chloride (Sigma–Aldrich, St. Louis, MO), stannous chloride (Sigma–Aldrich, St. Louis, MO), magnesium chloride (Sigma–Aldrich, St. Louis, MO), lead acetate (Fisher, Pittsburgh, PA), lithium chloride (Fisher, Pittsburgh, PA), ferric chloride (Fisher, Pittsburgh, PA), lanthanum chloride (Fisher, Pittsburgh, PA), phenol (Sigma–Aldrich, St. Louis, MO). All chemicals listed here are reagent grade or higher.

The following chemicals were reacted with mercuric chloride to investigate the precipitation reaction: Cremophor® EL (BASF, Dortmund, GER), polyethylene glycol MW 400 (Mallinckrodt, St. Louis, MO), ethylene glycol dimethyl ether (Sigma–Aldrich, St. Louis, MO), castor oil (Fisher, Pittsburgh, PA), triglyme (Sigma–Aldrich, St. Louis, MO), glycerol ethoxylate (Sigma–Aldrich, St. Louis, MO), *cis*-3-hexene-1-ol (Sigma–Aldrich, St. Louis, MO). All chemicals listed here are reagent grade or higher.

The following solvents were used in NMR studies: ethanol (Sigma–Aldrich, St. Louis, MO), deuterated acetone (Sigma–Aldrich, St. Louis, MO), deuterated water (Sigma–Aldrich, St. Louis, MO). The solvents used here were analytical grade reagents (>99.9%).

The following solvents were used in the IR studies: mineral oil (Nujol, Sigma–Aldrich, St. Louis, MO), ethanol (Aaper, Shelbyville, KY). All solvents were analytical grade reagents.

The following solvents were used in the HPLC studies: acetonitrile (Burdick and Jackson, Muskegon, MI), ethanol (Aaper, Shelbyville, KY), water (Milli-Q, Waters, Milford, MA). All solvents used were chromatographic grade. Paclitaxel (Hauser Chemical, Boulder, CO), Cremophor® EL (BASF, Dortmund FRG) and 13-taxanes mixture (Hauser Chemical, Boulder, CO) were injected as analytes.

### 2.2. Equipment

$^{1}H$ and $^{13}C$ NMR spectra were acquired on a Bruker Avance DRX400 multinuclear spectrometer. All the compounds used in the NMR analysis were dissolved and analyzed in $d_6$-acetone. The spectra were acquired and processed with XWINN-NMR® software.

Infrared absorption spectra were obtained with a Mattson Genesis Series Fourier FTIR spectrophotometer and then processed with Winnfirst® software.

The HPLC components used were a Hewlett-Packard 1100 autosampler, Hitachi L-7100 quaternary gradient pump, and a Waters 2487 dual wavelength absorbance detector. A Waters 717 Plus autosampler was also used with the components listed above. The column used throughout the HPLC studies was a Phenomenex Curosil, 250 mm × 4.6 mm, with a 5 μm particle size. The column was maintained at ambient temperature. Separations were achieved using 40% acetonitrile in water for mobile phase A and 70% acetonitrile in water for mobile phase B. A gradient elution was performed where after 25 min, mobile phase A was reduced from 100 to 10% at 60 min. This was followed by a column wash of 100% mobile phase B for 10 min. The later step in the gradient was necessary to elute compounds attributed to CrEL. The flow rate was set at 1.0 mL/min and the detection wavelength used was 228 nm. The injection volume was 10 μL. All chromatographic data was acquired and processed with Waters Millennium® 4.0 software.

### 2.3. Precipitation reaction

Table 1 shows a list of compounds that were screened for a possible reaction and precipitation with CrEL. Since mercuric

Table 1
Compounds screened for insoluble precipitate reaction with Cremophor® EL

| Compound | Insoluble precipitate formation |
|---|---|
| Similar ionic radii | |
| Mercuric chloride | Yes |
| Mercuric iodide | No |
| Mercuric bromide | Yes (very slight) |
| Barium chloride | No |
| Mercuric acetate | Yes (very slight) |
| Phenolic hydroxy compounds | |
| Resorcinol | No |
| Phenol | No |
| Chloride salts | |
| Ferric chloride | No |
| Sodium chloride | No |
| Lanthanum chloride | No |
| Cobalt chloride | No |
| Copper chloride | No |
| Zinc chloride | No |
| Nickel chloride | No |
| Silver chloride | No |
| Stannous chloride | No[a] |
| Magnesium chloride | No |
| Other | |
| Lead acetate | No |

[a] Silver chloride sparingly soluble in ethanol.