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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.

Table 2
Compounds screened in the study of functional group interaction with Cremophor® EL

Compound	Representative functional group	Insoluble precipitate formation
cis-3-Hexen-1-ol	Alkenol	No
2-Heptene	Alkene	No
1-Octene	Alkene	No
Castor oil	Aliphatic Chain	No
Glycerol ethoxylate, $n=6-7$	Glycerol backbone and ethers	Yes
PEG 400	Ether	Yes
Diglyme	Ethylene	No
Triglyme	Ethylene	Yes

chloride was known to form an insoluble precipitate with CrEL [1], most other compounds attempted were of similar ionic radii, or were chloride salts. Additionally, two organic compounds thought to form an insoluble complex were also attempted [1]. Saturated solutions containing each of these compounds were prepared and mixed with a solution of 50% CrEL in ethanol.

Table 2 lists other compounds that replaced CrEL in a reaction with mercuric chloride in order to study the complex formed by the precipitation. Each of these compounds possesses a functional group found in CrEL that may interact with mercuric chloride to form an insoluble complex. The complexes that were isolated using these compounds were examined by NMR and FTIR to assist in the elucidation of the precipitation mechanism.

The precipitation reaction was performed by diluting the CrEL blank or analytical sample in a solution of ethanol saturated with mercuric chloride. After completion of the precipitation, the solution was centrifuged at 5000 rpm for 10 min and filtered using a 0.45 μm borosilicate glass hydrophilic polypropylene (GHP) filter (Gelman Acrodisc). The supernatant was then analyzed via HPLC. The precipitate collected was transferred to a Buchner funnel, washed with ethanol and dried over silica gel, under vacuum, for three days. Similar reactions were performed on triglyme and polyethylene glycol (PEG 400) in order to collect precipitate for elucidation of the precipitation mechanism.

2.4. HPLC analysis

Stock solutions of paclitaxel were prepared by dissolving paclitaxel at 25 mg/mL in a solution of 50% CrEL in ethanol. For the recovery study, a recovery stock solution was prepared containing paclitaxel at a concentration of 25 mg/mL in a solution of 50% CrEL in ethanol and spiked with a mixture of 13 related substances of paclitaxel, each at 1.0% of the nominal analytical concentration (0.5 mg/mL). The precipitation reaction was then performed on the blank, sample and recovery solutions by making a 1:50 dilution (0.5 mg/mL paclitaxel) in an ethanol solution saturated with mercuric chloride. A control sample containing paclitaxel at 0.5 mg/mL without CrEL was prepared and spiked with the 13 taxane mixture at 1.0%. The HPLC analysis was carried out using the conditions described above. The percent recovery was calculated with the peak areas of the sample and control solutions.

2.5. NMR analysis

The precipitates from the reaction of mercuric chloride with both CrEL and PEG 400 were analyzed via ^{1}H NMR by dissolving in d_6-acetone at 20 mg/mL. Samples of unreacted CrEL and PEG 400 were prepared in a similar manner. Analysis via ^{13}C NMR was performed on both CrEL and PEG 400 in the same manner but at a concentration of 50 mg/mL. The d_6-acetone peak was used for calibration (2.04 ppm for ^{1}H NMR spectra and 29.94 ppm for ^{13}C NMR spectra).

2.6. FTIR analysis

Two milligrams of the CrEL precipitant was triturated intimately with four drops of mineral oil (nujol) in a mortar. The mixture was suspended between two sodium chloride plates and the spectrum was obtained in a blank subtracted background. Two milligrams of the triglyme precipitant was prepared similarly. The spectrum of the unreacted CrEL and triglyme were also obtained under the same conditions as the precipitant.

3. Results and discussion

3.1. HPLC assay results

Studies were carried out to determine if the precipitation reaction described above would interfere with the quantitation of paclitaxel and related substances. Injections of a solution containing paclitaxel and CrEL were made on an HPLC system using the conditions outlined above. Fig. 1 shows the large number of peaks attributed to CrEL. A chromatogram of the CrEL solution after precipitation via mercuric chloride shows a dramatic decrease in the number of peaks attributed to CrEL. Several peaks remain that are attributed to the CrEL, however none of these peaks interfere with the quantitation of the impurities associated with paclitaxel. As this technique does not remove every peak associated with CrEL, individual evaluation of each drug product for specificity with regards to remaining CrEL peaks must be performed.

Accuracy by recovery was conducted using a paclitaxel sample in which the CrEL was removed by precipitation with mercuric chloride. This was performed to demonstrate the specificity of the precipitation reaction towards CrEL. Fig. 2 shows the chromatographic overlay of a standard containing the 13 related substances and a spiked sample in which the CrEL was removed. The repeatability and recovery results (see Table 3) showed that the precipitation of CrEL by mercuric chloride does neither involve paclitaxel nor related substances.

3.2. Elucidation of precipitation reaction

Mercury(II) chloride was shown to be the only compound to elicit a strong complexation and precipitation with CrEL

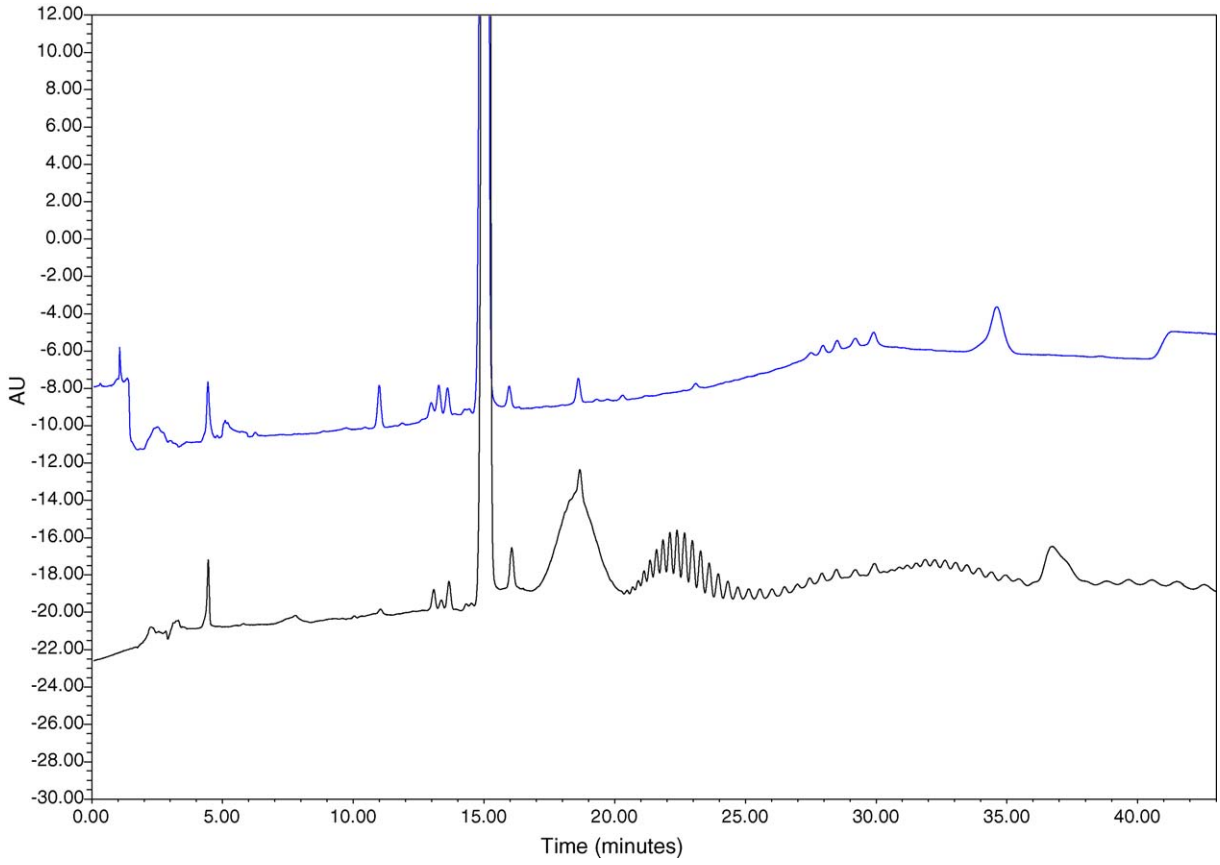

Fig. 1. Chromatographic overlay of paclitaxel after complexation with mercuric chloride (top) and in the presence of Cremophor® EL (bottom).

in an ethanol media. Mercuric bromide and mercuric acetate elicited weak but present precipitation. Previous investigation indicates the stability of the CrEL–metal complex in ethanol is strongly related to the size of the metal ion being chelated [7]. Metals, including mercury, that have an ionic radius greater than 1.0 Å will strongly bind to CrEL. The metal ion must also maintain its bonds with the cation to form a neutral ethanol-insoluble polymer. Based on the hard/soft acid base principle, mercury(II) is a soft acid that strongly binds chloride ions [8]. Mercuric bromide and mercuric acetate form complexes with CrEL which are only partially soluble due to the weaker bonding of mercury with bromine and acetate.

Table 3
Accuracy by recovery results for paclitaxel related substances

Number	Name	Retention time (min)	Peak area	%Recovery
1	10-Deacetylbaccatin III	4.90	11520	111
2	Baccatin III	7.23	7679	92
3	7-Xylosyl-10-deacetyl-taxol B	8.01	10092	116
4	Taxinine M	8.31	9359	94
5	7-Xylosyl-10-deacetyl-taxol	9.14	6136	111
6	Unknown degradation peak	10.37	4352	90
7	7-Xylosyl-10-deacetyl-taxol C	10.67	9198	100
8	7-Xylosyl-taxol	11.35	14292	117
9	10-Deacetyl-taxol	13.71	8820	113
10	Cephalomannine (taxol B)	14.05	26271	126
11	7-Epi-10-deacetyl-taxol	14.75	16160	112
12	Taxol	15.52	10753090	110
13	Taxol C	16.68	6806	99
14	7-Epi-taxol	19.19	28873	108

NMR and FTIR analysis performed on the isolated precipitate indicate that the complexation involves the ether linkages of the CrEL and not the olefin or glycerol backbone. Compared with NMR spectra of raw CrEL, the ^{1}H NMR spectra of the mercuric chloride–CrEL complex contained several chemical shifts (see Fig. 3). The most notable shift was at approximately 3.6 ppm, an area associated with the hydrogens adjacent to the electronegative ether oxygens. A strong downfield shift was seen. This downfield shift is indicative of a removal of electron density surrounding the nuclei of the ether protons, as would be the case in the presence of mercury. A second change in the NMR spectra is attributed to the water present in the CrEL raw material. This change occurs at approximately 2.8 ppm and was elucidated by deuterium substitution. No change in the chemical shifts of alkene protons, methylene protons or glycerol fatty acid protons are observed.

The ^{13}C NMR spectra of CrEL and the mercuric chloride–CrEL complex provided further support to the results from the ^{1}H NMR studies. The chemical shifts of carbons adjacent to the oxygens, generally occurring between 80 and 40 ppm, illustrate the region where there was significant shifting when mercuric chloride is complexed with CrEL. The chemical shifts are upfield in the spectrum of the mercuric

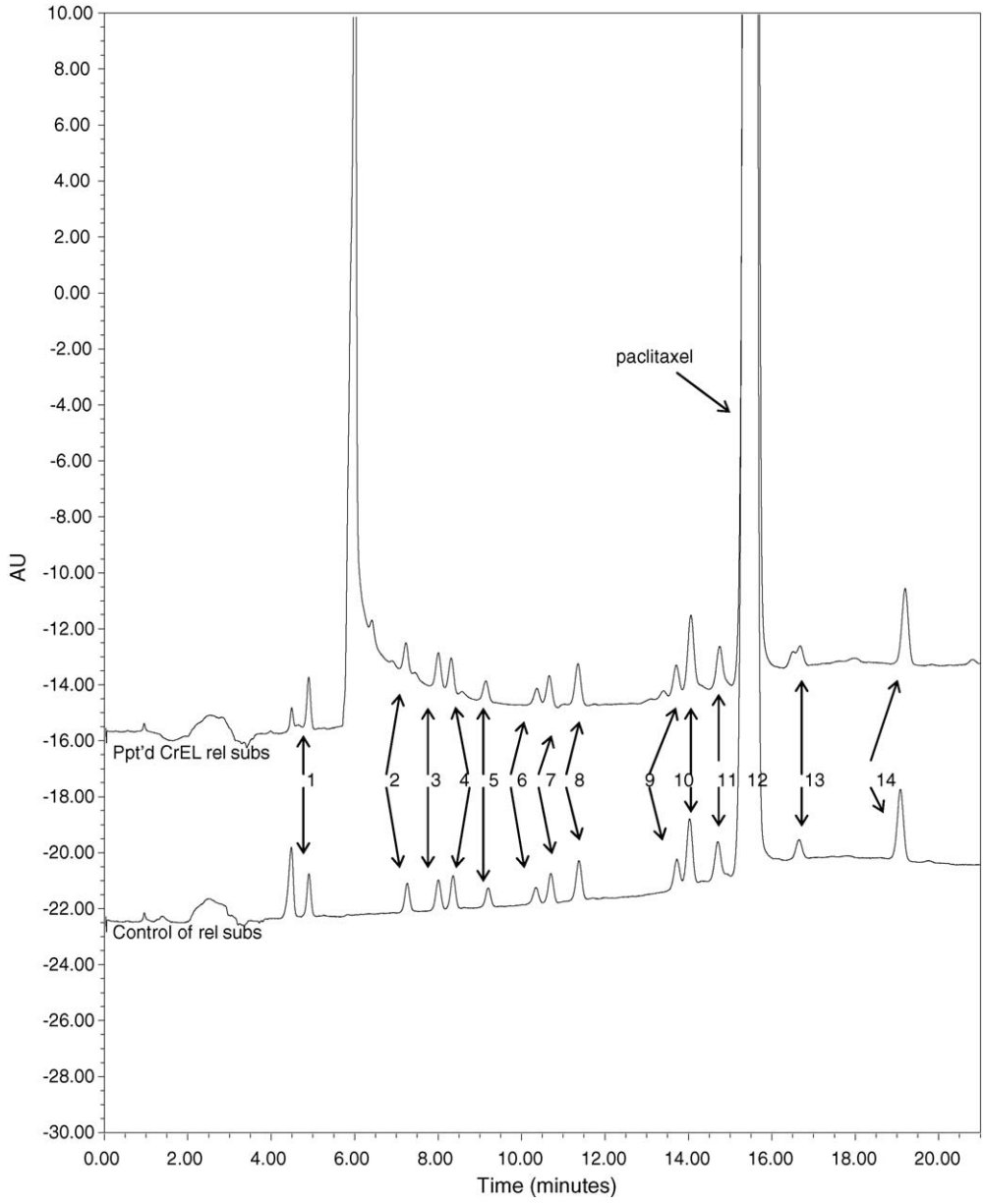

Fig. 2. Chromatographic overlay of control solution (bottom), and recovery solution after precipitation with mercuric chloride (top).

chloride complexed CrEL, alluding to a shielding effect on the carbons adjacent to the ether oxygens present (Fig. 4). The carbonyl carbons of CrEL, which absorb at approximately 174 ppm, did not show any effect when mercury is complexed. As expected, there was no noticeable shifting in the region where the alkane carbons absorb (40–20 ppm). The difference in shift vector between the ^{1}H NMR and ^{13}C NMR for the ether elements was noted, but the cause is unknown.

FTIR was used to investigate the vibrational changes in certain functional groups present in CrEL and the mercuric chloride complexed CrEL. A notable difference between the two spectra existed in the ether (1100 cm^{-1}) and ester (1730 cm^{-1}) absorption regions. The carbon–oxygen bending due to the ether linkages at 1100 cm^{-1} was substantially reduced when CrEL is complexed with mercuric chloride. The ester peak found at 1730 cm^{-1} also showed a significant decrease is absorption when CrEL is complexed with mercuric chloride (see Fig. 5). No change in absorption was observed for the alkene absorption regions (3020–3100 cm^{-1} and 1650–1670 cm^{-1}).

The results of the NMR and FTIR studies were further supported by examining precipitates formed when mercuric chloride was reacted with compounds that contain functional groups similar to those found in CrEL. Glycerol ethoxylate, PEG 400 and triglyme formed precipitates upon reaction with mercuric chloride. These compounds posses ether groups in common with CrEL. ^{1}H NMR spectra of the mercuric chloride–PEG 400 complex also showed the downfield shift at about 3.6 ppm associated with the shielding of ether-

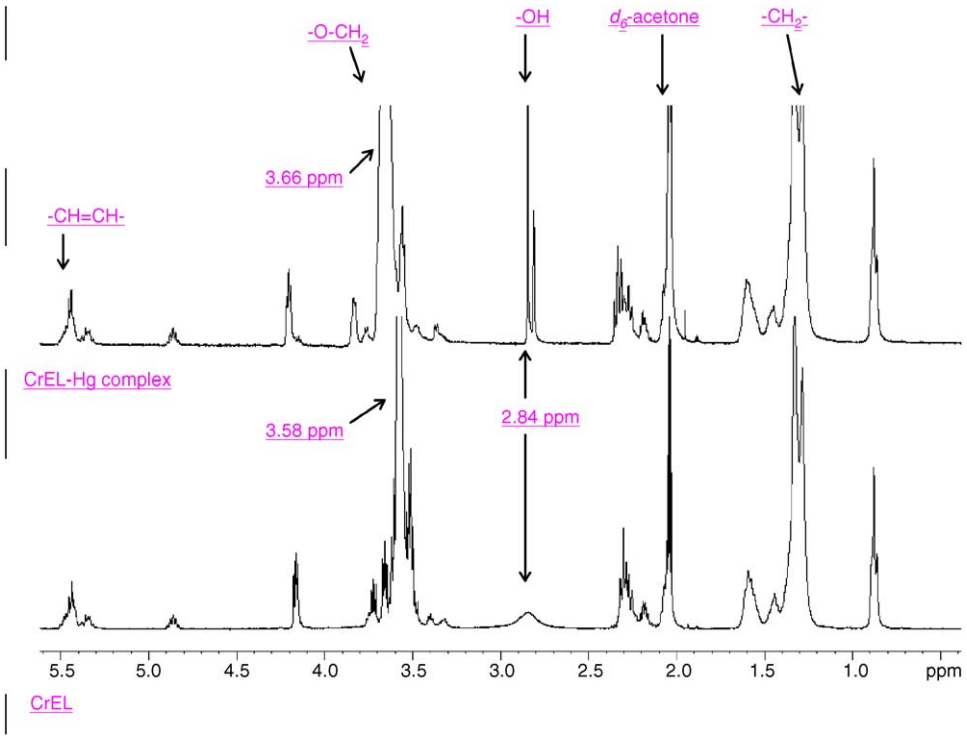

Fig. 3. ^{1}H NMR showing CrEL complexed with mercuric chloride (bottom) and raw CrEL. The downfield shift is shown at 3.6 ppm.

bonded proton by mercury. ^{13}C NMR spectra similar to the mercuric chloride–CrEL complex were also observed with the mercuric chloride–PEG 400 complex. The complexation of mercuric chloride to triglyme, triethylene glycol dimethyl ether, also supports the interaction of mercuric chloride with the ether linkages. The lack of complexation seen with diglyme (diethylene glycol dimethyl ether) is thought to be due to steric reasons. With only three coordinating ether oxygens,

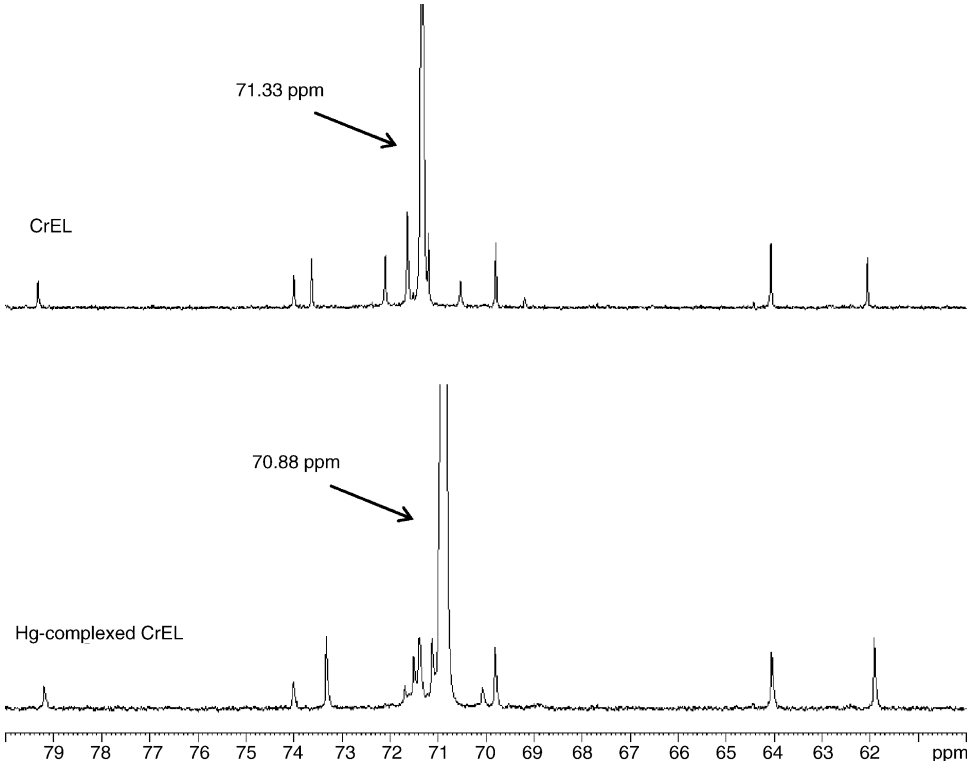

Fig. 4. ^{13}C NMR spectra illustrating the chemical shifts seen in when CrEL complexes with mercuric chloride (bottom).

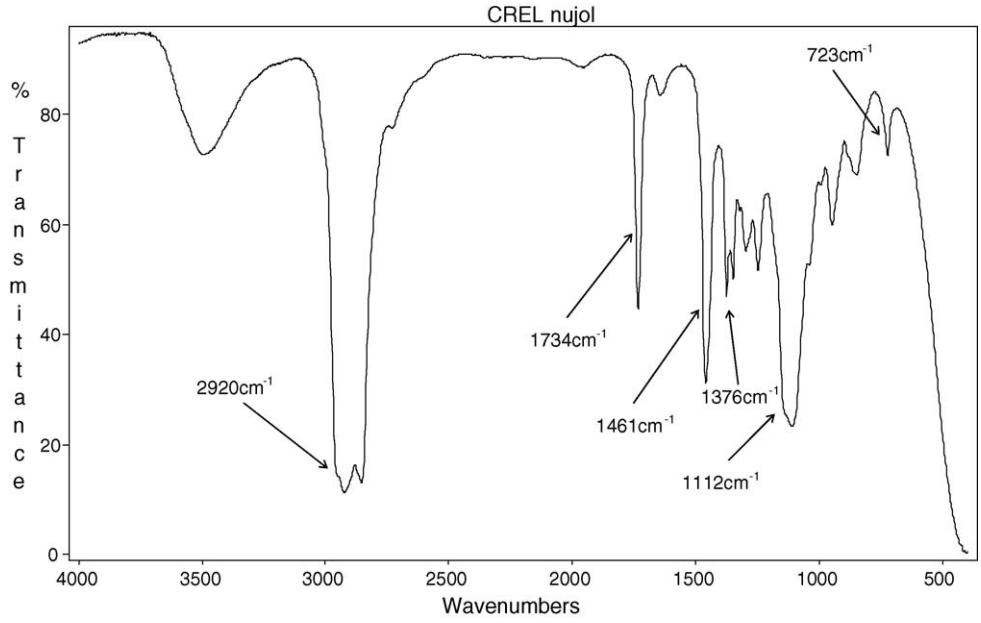

Fig. 5. FTIR spectra of CrEL (top) and CrEL complexed with mercuric chloride (bottom).

diglyme lacks the ability to form a tetrahedral structure capable with triglyme [9]. FTIR spectra of mercury complexed with triglyme resemble that of mercury complexed with CrEL in that the region of ether bonding (1100 cm^{-1}) was substantially reduced.

4. Conclusion

A novel technique for the removal of CrEL in order to accurately quantitate paclitaxel and related substances by HPLC with UV detection is presented. The accuracy studies performed show that no discernable amount of paclitaxel or any of the 13 related substances examined were lost during the precipitation reaction. The removal of CrEL is via the formation of an insoluble complex with mercuric chloride. The analysis of the mercury-complexed CrEL via NMR and FTIR support the theory that CrEL is selectively removed from a solution by divalent mercury binding to the polyether moiety of the CrEL. The application of this technique to other drugs formulated in a similar manner is dependent on the presence and location of the polyether moiety.

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