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Determination of surface-bound hydroxypropylcellulose (HPC) on drug particles in colloidal dispersions using size exclusion chromatography: A comparison of ELS and RI detection

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Abstract

Evaporative light scattering (ELS) and refractive index (RI) detection methods were evaluated for the determination of surface-bound hydroxypropylcellulose (HPC) on drug particles in colloidal dispersions. Size exclusion chromatography (SEC) was used to separate HPC from other components of the dispersions. The instrumental parameters of the ELS detector were optimized to obtain maximum peak intensity, adequate peak shape and minimal baseline noise by varying the mobile phase flow rate, nebulizer temperature, and evaporation temperature. The chromatographic method was validated using both detectors. The ELS detector response exhibited second order polynomial and linear double logarithmic correlation with concentration over a 10–300% range while the RI response was linear. The double logarithmic correlation simplified the calculation compared to using the polynomial fit, and it provided more accurate results compared to the linear fit approach. Total HPC was obtained by solubilizing all components of the dispersion and analyzing for HPC. Non-bound HPC was obtained by ultracentrifuging the dispersion and analyzing the supernatant for HPC concentration. Analysis for total- and non-bound HPC in a representative colloidal dispersion gave method precisions with R.S.D.s of 2.5 and 2.2% for ELS, and 4.5 and 2.4% for RI ($n = 4$). HPC bound to the surface of the drug particles was determined by difference: % bound HPC = 100% − % non-bound HPC. Resultant % bound HPC values ranged from 22.1 to 25.4% of available HPC. Both ELS and RI are satisfactory detection techniques for HPC quantitation and for determination of the proportion of HPC bound to drug colloid particles, and the assay results are comparable.

Keywords: Surface-bound; Hydroxypropylcellulose (HPC); Evaporative light scattering detection (ELSD); Refractive index (RI); Size exclusion chromatography (SEC); Colloidal dispersions

1. Introduction

An increasing number of newly developed drugs are poorly soluble in water, which is a general problem in pharmaceutical drug formulation [1]. Typical problems associated with poorly soluble drugs are low bioavailability and erratic absorption [2]. Producing colloidal dispersion formulations containing drug nanoparticles for poorly soluble drugs is an alternative and promising approach. The main advantages of colloidal dispersions are their increase of saturation solubility and dissolution rate, improving the bioavailability of drug [3]. Another feature of colloidal dispersions is that nanoparticles have general adhesiveness to the intestinal wall, which leads to a prolonged residence and contact time in the gastrointestinal tract (GIT) [4,5]. A common approach for producing nanosuspensions is high pressure homogenization developed by Müller et al. [6]. However, during the storage of nanosuspensions, problems including sedimentation, caking, and flocculation often occur due to strong van der Waals attractions [3]. To control the intrinsic tendency of the colloidal particles to flocculate, polymers are often used to stabilize the dispersion [3]. The polymers avoid each other and, provided that the repulsive forces have a longer range than the attraction forces, the particles will be kept apart. This is so-called "steric stabilization" [7].

Polymers such as various cellulose ethers, including methylcellulose (SM-25 and SM-100) and 2-hydroxypropyl methyl-

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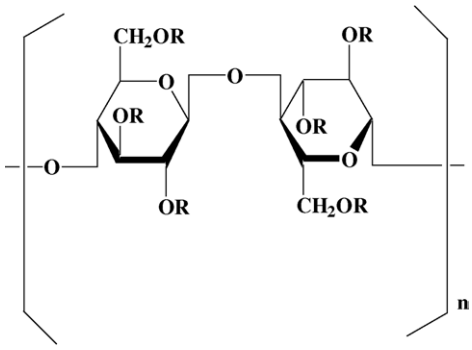

R is H or $[\text{-CH}_2\text{-CH(CH}_3\text{)-O}]_m\text{H}$

Fig. 1. Structure of HPC.

cellulose (SH-50), have been used as stabilization agents for the suspensions of two steroids with low aqueous solubility [8]. Cellulose SH-50 and SM-100 were used as stabilization agents for 5-(3-ethoxy-4-pentyloxyphenyl)-2,2-thiazolidinedione (CT112, an enzyme inhibitor) [9]. Since the high dispersion stability of colloids conferred by non-ionic polymers can be attributed to the steric repulsion between adsorbed polymer layers [9], assessing the percent polymer bound to the drug nanoparticles is an important parameter to characterize during the colloidal dispersion stability study. Adsorption of cellulose on drug particles is usually measured by a depletion method. The procedure usually includes the separation of drug particles from the solution (supernatant) either by ultrafiltration [11] or ultracentrifugation [8]. The separated drug particles are re-dissolved into solution, and the polymer concentrations in the supernatant and the other solution are determined to obtain the non-bound and total polymer amounts, respectively. A differentiation method is then used for the quantitation of cellulose (bound) on the particles.

Hydroxypropylcellulose (HPC) is a cellulose ether obtained by chemical reaction of the hydroxyl groups at positions 2, 3, and/or 6 of the glucose residues of cellulose (see Fig. 1 for structure). HPC is a commonly used drug excipient [10]. Recently, during our formulation development of a nano-colloidal dispersion formulation for a water-insoluble drug, HPC was used to sterically stabilize the drug colloidal dispersions containing drug and a dispersing agent. The extent of HPC surface-bound on drug particles (defined as percent HPC bound to the drug) can play an important role in the stability of the dispersion. The development of a simple and accurate method to determine surface-bound HPC plays a critical role in this endeavor. A widely used method for quantitation of HPC is to hydrolyze the alkoxyl groups with hydroiodic acid and quantify the resulting halogenated derivatives by redox titration; this is the USP assay method for HPC [12]. This method is tedious and time-consuming, and it is indirect as it assays for the hydroxypropoxy group, instead of the entire HPC molecule. A spectrophotometric method using Anthrone reagent to react with the polymer to produce a dark green solution was reported and used for determining the concentrations of cellulose ethers, sucrose, polysaccharides, and starch [13,14]. The method usually provided good sensitivity; however, it is not specific for HPC. For example, common dispersing agents for colloidal dispersions react with the Anthrone reagent and cause interference with HPC quantitation [15]. Also, the method requires the Anthrone reagent to be prepared 24 h prior to its use, which is inconvenient, in order to provide reproducible data [16].

Size exclusion chromatography (SEC) is commonly used for the separation and quantitation of polymers [17]. The determination of HPMC and PEG 400 in pharmaceutical formulations was reported previously [18,19]. SEC has also been used for the characterization of the molecular mass distribution range of HPC [19]. Refractive index detector (RI) is a universal detector [20], which can be used to detect analytes without UV chromophores, such as HPC. Recently, applications of evaporative light scattering detectors (ELS) have increased dramatically due to the possibility of replacing the RI detector and the greater flexibility of ELS [21,22]. ELS detection has been used to successfully quantitate synthetic polymers [23], carbohydrates [19,24], fats and fatty acid esters [25,26], triglycerides [27], and steroids [28]. However, even though both detection technologies are available, no methods have been reported using size exclusion chromatography wherein ELS and RI detection have been compared for quantitation of HPC. Operating principles and factors which may affect ELS detector responses have been reported, but little work has been done to demonstrate how the method development can be conducted practically. This paper reports the development and validation of a method for quantitation of HPC using SEC with simultaneous ELS and RI detection, to overcome the detection difficulty for an analyte without a UV chromophore, such as HPC. Effects of major instrumental parameters for ELS detection were explored and optimized. The simultaneous use of both detectors allowed the direct comparison of the advantages and disadvantages of both detectors. The method was then used to determine the surface coverage of HPC in a drug colloidal dispersion.

2. Experimental

2.1. Chemicals and reagents

HPC (MW 64,000–92,000 Da, viscosity 3.0–5.9) was obtained from Nippon Soda Co. Ltd. (Tokyo, Japan). HPLC grade DMF and ammonium acetate were obtained from Fisher Scientific (Pittsburgh, PA, USA). Dispersing agent S was obtained from Tate and Lyle (Brooklyn, NY, USA). The solid materials were stored at room temperature in darkness. A colloidal dispersion formulation of Merck compound A was provided by Merck Formulation Design group. The formulation consists of 48 mg/mL drug, ∼1.255% HPC and ∼3.9% dispersing agent (refer to as S below).

Mobile phase was prepared by dissolving various amounts of ammonium acetate (0–0.04 M) in dimethyl formamide (DMF). Method diluent was prepared to contain 0.02 M ammonium acetate in DMF. A 100% standard solution of HPC was prepared by dissolving ∼25 mg of the solid in 100 mL method diluent by sonication and stirring.

2.2. Size exclusion chromatography and detection

The separation of HPC from the other components of the formulation was by size exclusion chromatography. The separation columns, two PLgel MIXED-E (300 mm × 7.5 mm), 3 μm particle size connected in series, were purchased from Polymer Laboratories (Amherst, MA, USA). The HPLC system was a Waters Alliance 2690 Separation Module (Waters Corporation, Milford, MA, USA), including vacuum degasser, pump, autosampler and column heater.

The ELSD Model PL-ELS 1000 was manufactured by Polymer Laboratories, Inc. (Amherst, MA, USA). The refractive index detector was manufactured by Perkin Elmer (Model 200 Series, Perkin Elmer, Wellesley, MA, USA).

The optimized chromatographic conditions are listed below:

Run time	30.0 min
Detectors	ELSD and RI
Mobile phase	0.02 M ammonium acetate in DMF
Flow rate	0.8 mL/min
Column temperature	60 °C
Injection volume	50 μL
ELSD conditions	
Nebulizer temperature	100 °C
Evaporation temperature	180 °C
N_2 gas flow	1.5 mL/min

2.3. Sample preparation and calculations

The procedure for separating bound and non-bound HPC in the drug colloidal dispersions is illustrated in Fig. 2. These procedures were modified from the procedures developed by Booth et al. [29]. About 1800 mg ($W1$, mg) of the dispersion was transferred to a pre-weighed Beckman polycarbonate centrifuge tube (13 mm × 56 mm). The dispersions were ultracentrifuged using a Beckman Ultracentrifuge (Model Optima™ Max, Beckman Coulter, Inc., Fullerton, CA, USA) at 110,000 rpm for 2 h. The separation of supernatant and the "drug pellet" was observed. The non-bound HPC, which resided in the supernatant, was thus separated from the bound HPC adsorbed by the drug, which resided in the pellet. The entire supernatant (e.g., about 1600 mg) was removed and weighed ($W2$, mg). About 385 mg of the supernatant ($W3$, mg) were weighed into a 10 mL volumetric flask and diluted to volume using the method diluent. External standards of HPC ranging from 50 to 150% of the method concentration were injected along with the samples to provide the calibration curve for both detectors. The concentration of HPC in the diluted supernatant solution was then obtained (C_s, in mg/mL). The HPC concentration in the undiluted supernatant (non-bound HPC) was calculated using the following equation:

$$\text{HPC concentration in supernatant (mg/g supernatant)} = C_s \times 10\,\text{mL} \times \frac{1}{W3}$$

The HPC concentration in colloidal dispersion that had not been centrifuged was then determined. About 195 mg ($W4$, mg) of the dispersion was completely dissolved in 10 mL of diluent and then assayed by SEC with both detectors, which gave C_d. The total HPC concentration in the dispersion (undiluted) was

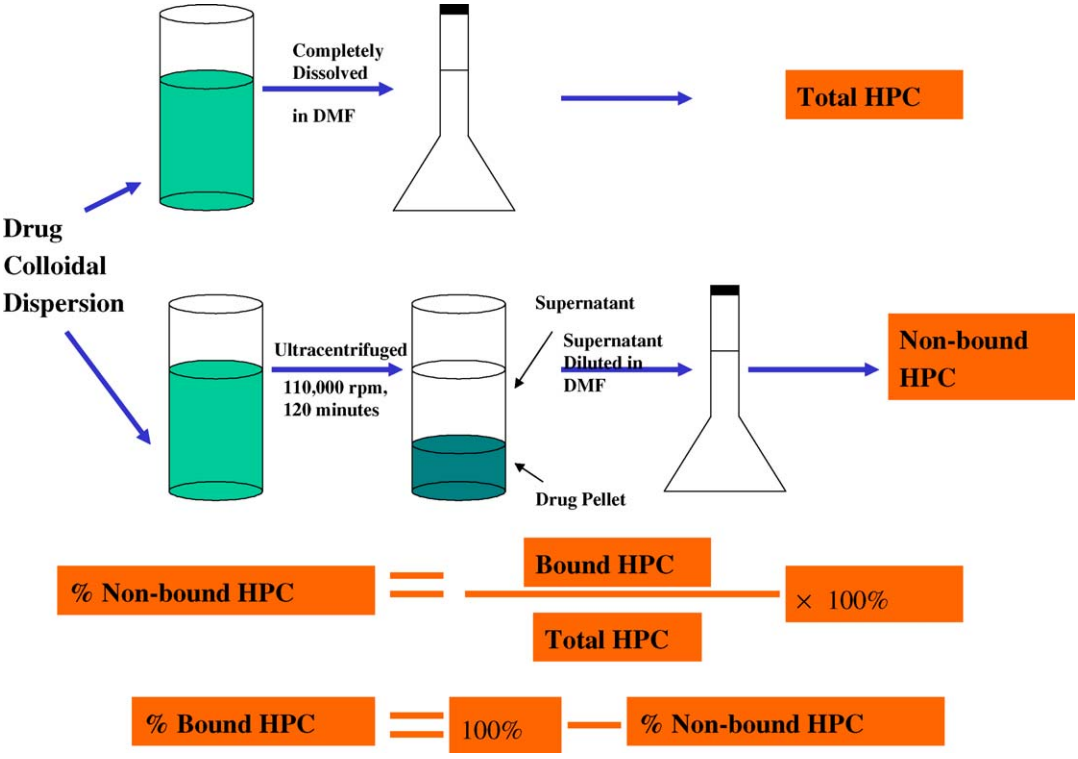

Fig. 2. Scheme for the determination of bound and non-bound HPC in the drug colloidal dispersions.

calculated as:

$$\text{Total HPC concentration in dispersion (mg/g dispersion)} = C_d \times 10\,\text{mL} \times \frac{1}{W4}$$

The percentage of HPC not bound to the drug (% non-bound HPC) was calculated as the ratio of HPC in the supernatant to total HPC in the dispersion, as shown in the equation below:

$$\%\,\text{Non-bound HPC} = \frac{\text{HPC concentration in supernatant} \times W2}{\text{Total HPC concentration in dispersion} \times W1} \times 100\%$$

The % bound HPC was calculated by subtracting the % non-bound HPC from 100%.

3. Results and discussion

3.1. Selection of chromatographic conditions

Previously, a SEC method was developed for HPC by Booth et al. [29] using the PLgel MIXED-E column and 0.1% lithium bromide in DMF as mobile phase. This method served as our starting point. Since the lithium bromide used in the mobile phase was not compatible with the ELS detector, it was replaced with ammonium acetate, which is volatile and thus compatible with ELS detection. The ionic strength effect was evaluated for the size exclusion separation to optimize the concentration of ammonium acetate. The other chromatographic conditions in the method were also modified in order to optimize the performance of the ELSD. The detection mechanism of ELSD is illustrated in the diagram shown in Fig. 3 [30]. When HPLC eluents pass through the ELSD, three main processes occur successively inside the detector. These are (1) nebulization of the chromatographic eluent to form fine droplets; (2) evaporation of the mobile phase droplet cloud to obtain particles; and (3) detection by light scattering from the residual particles, which comprise the analytes of interest [22]. Hence, the detection is

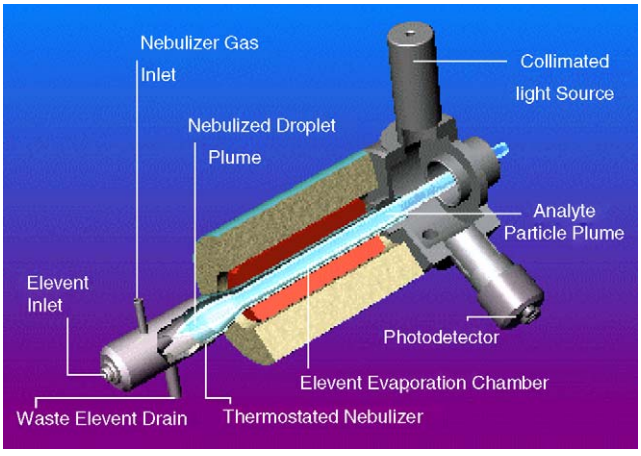

Fig. 3. Evaporative light scattering (ELS) detector (figure courtesy of Polymer Laboratories).

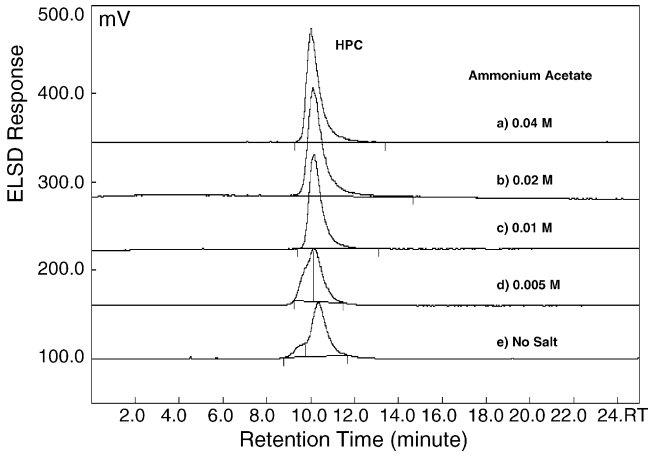

Fig. 4. Effect of mobile phase ionic strength on the peak shape of HPC.

affected by the following major factors: nebulizer temperature, eluent flow rate, and vaporization temperature. Therefore, these factors were evaluated. Since the operation of the RI detector is relatively simple, no specific optimization was conducted. All experiments were performed by injecting HPC standard solution (~0.25 mg/mL in DMF) into the system using different conditions. The chromatograms shown were all generated by the ELS detector.

3.1.1. Effect of ionic strength in mobile phase

The effect of ionic strength of the mobile phase was evaluated by varying ammonium acetate from 0 to 0.04 M in the DMF mobile phase. HPC standard at ~0.25 mg/mL in DMF was injected for the evaluation. The results are shown in Fig. 4 from ELS detector, similar results were also observed from the RI detector (data not shown). When pure DMF was used as mobile phase without any salt additive, the peak shape of HPC was poor. Ammonium acetate at a concentration of 0.02 M was chosen as the final additive concentration in the mobile phase. Salts, such as LiBr, are often added to polar organic solvents such as DMF, DMSO, DMAc, and NMP to reduce aggregation during the analysis of polymers [31]. In our case, the presence of strong hydrogen bonds between components of the cellulose backbone structure could lead to aggregation of the samples in the solvent, resulting in the elution of the polymer from the column with artificially low retention time. In the worse cases, the polymers can be forced into interacting with the packing material of the column. This manifests itself as a broader peak shape. The ammonium acetate with the polar solvent helped to break the hydrogen bonds and therefore to minimize aggregation of the HPC [32].

3.1.2. Effect of mobile phase flow rate

The effect of mobile phase flow rate on the ELSD was evaluated from 0.7 to 1.0 mL/min (Fig. 5a). The flow rate was found to have some effect on the baseline noise by evaluating the average noise levels at five points across the chromatogram. A lower flow rate promoted more complete evaporation of the mobile phase in the ELSD, thus reducing the baseline noise. However, decreasing the flow rate increased the run time. A flow

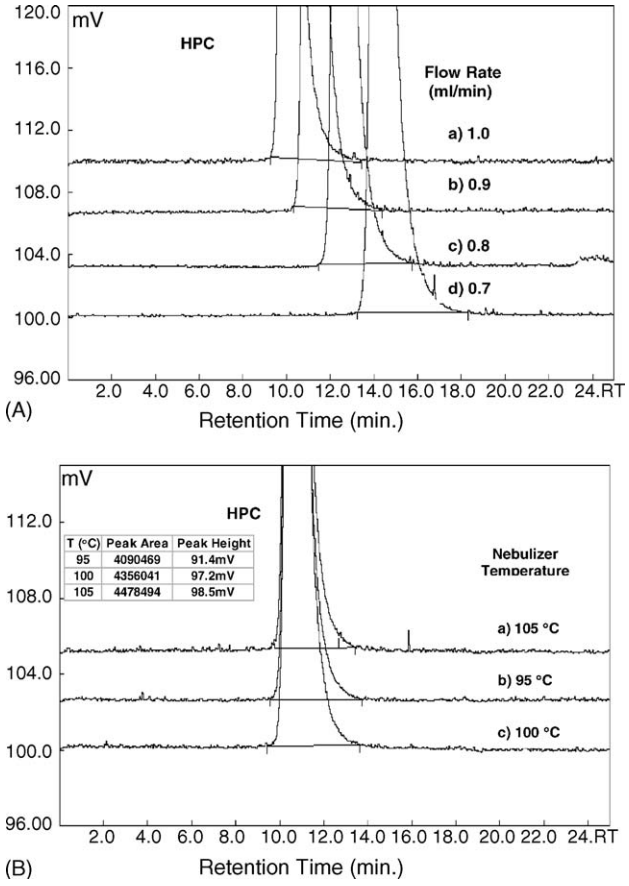

Fig. 5. Effects of mobile phase flow rate (panel A) and ELS detector nebulizer temperature (panel B) on the baseline noise of the ELS chromatograms.

rate of 0.8 mL/min, which provides better baseline noise with a reasonably short run time, was chosen as the final method condition.

3.1.3. Effect of nebulizer temperature and evaporation temperature

The variation of nebulizer temperature from 95 to 105 °C was evaluated for its effect on the baseline from the ELSD. Both 95 and 100 °C provided better baseline compared to 105 °C (Fig. 5b). However, the results show that increasing the nebulizer temperature increased the peak area and peak height of HPC. Therefore, the final condition was set at 100 °C. Evaporation temperatures of 165, 170, and 180 °C were evaluated and the temperature of 180 °C provided the optimum conditions for evaporation of the eluent, evidenced by the better baseline and peak shape compared to the other temperatures.

3.2. Method validation

Method validation was conducted to evaluate the performance of the method using the finalized chromatographic conditions for both RI and ELS detection. Validation elements included method specificity, detector response, recovery, measurement precision, repeatability, and sensitivity.

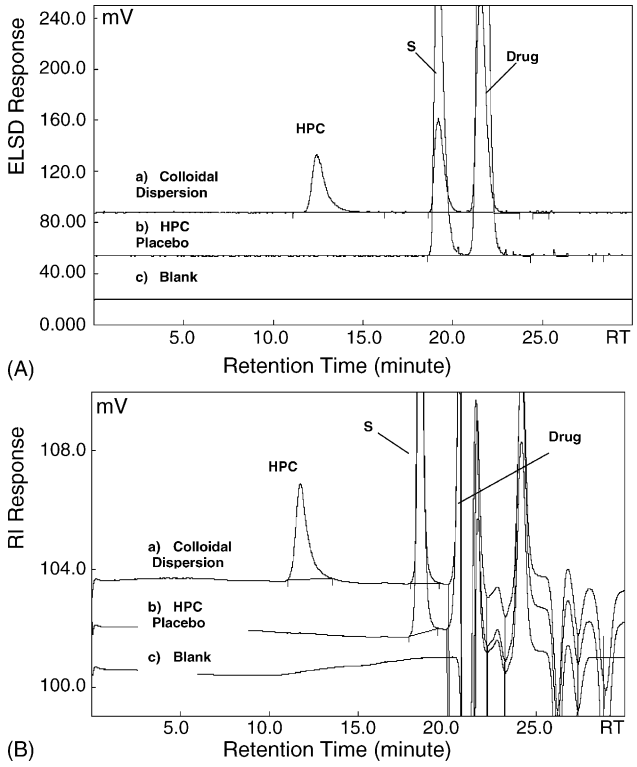

Fig. 6. ELS (panel A) and RI (panel B) chromatograms showing separation of HPC from the other components of the colloidal dispersion: dispersing agent S and the drug.

3.2.1. Specificity

Injections of diluent blank, HPC placebo solution (all compositions included in the colloidal dispersion except HPC) and the colloidal dispersion sample were conducted using both ELSD and RI detector (Fig. 6). There was no peak found in the diluent blank injections, and the placebo did not show any interference with the HPC peak. HPC in the formulation was separated from the drug and other excipients in the colloidal dispersion. The elution order of the peaks followed the size exclusion mechanism, i.e., larger molecules eluted first. Dispersing agent S and the drug were baseline separated using the ELS detector; therefore, potentially, the method can be used for the simultaneous determination of HPC, dispersing agent S and the drug. The RI detector can be used for the detection of dispersing agent S; however, the drug co-elutes with small solvent molecules and thus cannot be quantitated. The solvent molecules did not interfere in the ELS detection because they are volatile and thus were not detected.

3.2.2. Linearity

The linearity of the responses for both ELS and RI detectors was examined by spiking five different levels of HPC from 50 to 150% of the method concentration (0.25 mg/mL) into the placebo formulation (all other components except HPC). The RI detector exhibited linear response as a function of HPC concentration, with R^2 of 0.9990 (linear equation: $y = 407{,}212x - 9204$); however, the ELS detector response was only approximately linear, with R^2 of 0.9896 (linear equation: $y = 5{,}846{,}984x - 444{,}430$), and an evident non-linearity was

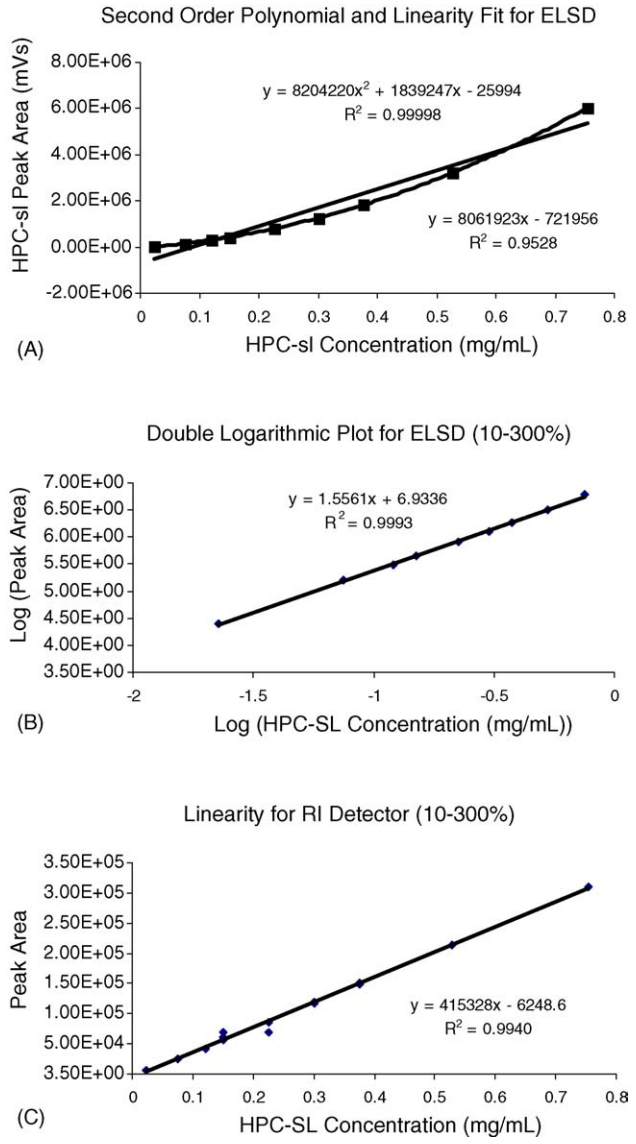

Fig. 7. Comparison of detector responses as a function of HPC concentration ranging from 10 to 300% of the method concentration (0.25 mg/mL). Panels A and B show the second order polynomial, linear, and double logarithmic fits of the ELS detector response; panel C shows the linear fit of the RI detector response.

observed. In order to optimize the accuracy for quantitation, a wider range of method concentrations was investigated in order to model non-linearity.

In a wider range from 10 to 300% of the method concentration, the ELS response is not linear ($R^2 = 0.9528$, Fig. 7a); however, the ELS response has an excellent second order polynomial fit ($R^2 = 0.99998$, Fig. 7a). Due to the nature of ELS detection, a sigmoidal calibration curve with a very nearly linear section in the middle tends to be generated [21]. The decline in sensitivity at the lower concentration end of the range is probably due to the predominance of the Mie scattering light deflection mechanism [21]. The decrease in sensitivity at the high concentration is due to the reduction in the surface ratio of the particles to the particle concentration, which causes a proportionally smaller amount of light to be reflected and refracted as the mass of each particle increases [21]. In the range we evaluated, the concentration (limited by the solubility) was not high enough to observe the full sigmoidal shape.

Since using a second order polynomial fit presented tedious data processing for quantitation, another approach using a double logarithmic fit was evaluated. When the eluent is nebulized at constant mobile phase and carrier gas flow rates, the size distribution of the eluent droplets remains constant. It does so also during the elution of the analyte, if the surface tension of the solution does not change significantly. During the vaporization of the solvent, the droplets shrink and their final volume is proportional to the analyte concentration. Then the response of the ELS detector is given by Eq. (1) [33]. In the event that the detector response is linear as a function of sample concentration, the constant b in Eq. (1) is equal to 1.00.

$$Y = a \times m^b \quad (1)$$

where, Y is the response of ELS detector; a the constant; b the constant; and m the mass or concentration of the sample.

This results in a linear relationship between the logarithm of the peak area and the logarithm of the sample concentration ($\log Y = b \times \log m + \log a$). This is confirmed by our results as shown in Fig. 7b of the double logarithmic plot for HPC concentration ranging from 10 to 300% of the method concentration (R^2 of 0.9993). The constants (b and a) were determined from the slope and the interception of the plot. In this case, the values for b and a are 1.56 and 8.58E + 6, respectively, indicating that the response is not linear as a function of sample concentration ($b \neq 1.00$). Compared to the polynomial fit, a double logarithmic plot simplified the data processing and was more practical for application to the assays. Thus, it was used for the quantitation of a wider range of HPC concentrations. While the ELS detector responded non-linearly from 10 to 300% method concentration, the RI detector response remained linear (Fig. 7c) over this range ($R^2 = 0.9940$).

3.2.3. Recovery

Recovery of HPC from the formulation matrix was conducted by spiking known amounts of HPC (50–150% of method concentration of 0.25 mg/mL) into the matrix. The results (Table 1) show that the average recoveries were 100.4% using both ELS and RI detectors. The results from the ELSD have much less variation (1.8% R.S.D., $N = 5$) compared to those from the RI detector (4.8% R.S.D., $N = 5$).

Table 1
Recovery of HPC from Solution-spiked formulation matrix

	Recovery (ELS)	Recovery (RI)
Levels (%)		
50	99.7	93.2
70	103.4	106.6
100	99.4	101.3
120	100.7	101.7
150	98.7	99.4
Average	100.4	100.4
R.S.D. (%)	1.8	4.8

Table 2
Injection precision of the method

	Peak area of HPC (ELS)	Peak area of HPC (RI)
Number of injection		
1	1311791	90817
2	1299559	94277
3	1304984	89557
4	1314085	91658
5	1301677	88911
6	1311693	90916
7	1334837	88230
8	1330701	90177
9	1296770	86152
10	1276701	90932
Average	1308280	90163
R.S.D. (%)	1.3	2.4

3.2.4. Measurement precision

Measurement precision was conducted by making replicate ($N = 10$) injections of HPC standard solution (0.25 mg/mL) into the HPLC. The R.S.D.s for HPC peak area (Table 2) were 1.3 and 2.4%, respectively, from the ELS and RI detectors, indicating that ELSD has better measurement precision.

3.2.5. Repeatability

The repeatability of the method was evaluated by preparing four samples from the drug colloidal dispersions. About 195 mg of the drug colloidal dispersions were weighed into a 10 mL volumetric flask, and diluted to volume using 0.02 M ammonium acetate in DMF as diluent. Since the formulation contains 1.225% HPC, the resulting solutions had an HPC concentration of approximately 0.239 mg/mL. The HPC concentration in the sample solution is limited by the drug solubility in DMF. All of the components including drug were completely dissolved at this concentration. The samples were quantitated using HPC standards. A double logarithmic calibration curve generated from standard injections in the same run was used for the ELSD, while the linear calibration curve generated from the same set of standards was used for the RI detector quantitation. The average assays (Table 3) were 102.4% claim and 104.2% claim with R.S.D.s of 2.5 and 4.5%, respectively, from the ELS and RI detectors. The average assays from the two detectors are within 2% (absolute), indicating the quantitation of HPC is equivalent between these two detectors. However, the ELS detector exhibited better method precision.

Table 3
Method precision for determination of total HPC in drug colloidal dispersions

	HPC in dispersion (percent claim)	
	ELS	RI
Sample number		
1	100.6	102.9
2	101.2	98.2
3	101.6	109.0
4	106.3	106.8
Average	102.4	104.2
R.S.D. (%)	2.5	4.5

Table 4
Limit of quantitation for HPC using ELS and RI detectors

Detector	ELS	RI
LOQ (per injection)	2 μg	1 μg
Peak height at LOQ	2189 μV	175 μV
Average noise level	150–200 μV	10–20 μV

3.2.6. Limit of quantitation (LOQ)

LOQ was defined as the lowest concentration that provided a signal-to-noise ratio above 10. It was found that ELSD and RI had comparable LOQs of 1–2 μg per injection (Table 4). Even though the peak height of HPC from the ELS detector at the LOQ level is more than 10 times higher than that from the RI detector, the noise levels from the ELS detector were also proportionally higher, resulting in no improvement of LOQ compared to the RI detector. If the greater noise level in the ELS detector could be reduced, the ELS would be a much more sensitive detection method.

3.3. Application: determination of HPC surface coverage in drug colloidal dispersions

The validated method in conjunction with the sample preparation procedure for separating the bound and non-bound HPC was used to determine the HPC surface coverage in a drug colloidal dispersion. Assays were conducted in a replicate of four preparations to obtain the % bound HPC in a freshly prepared drug colloidal dispersion. The total amounts of HPC in the dispersion were determined as shown in the method repeatability section. The non-bound HPC was separated from the bound HPC and the drug pellet as described in the experimental section. The % bound HPC was obtained according to the method described in the experimental section. The results are shown in Table 5. The average % bound HPC was determined as 25.4 and 22.1% by ELS detector and RI detector, respectively. The difference between the assays is less than 3.4% (absolute), which is reasonable considering the propagated error induced from the different determination steps. The R.S.D.s calculated directly from the four determinations were 4.7 and 8.9%, respectively, again indicating the better method precision of ELS detection.

3.4. Comparison of ELS and RI detectors

The RI detector is a universal detector, while ELS is a quasi-universal detector because it is not suitable for high-volatility solutes. More method development work to optimize the detector performance was needed for the ELS detector; however, the ELS detector was found to equilibrate much faster than the RI detector and was not influenced by ambient conditions, such as temperature change in the room. There were no negative peaks found using the ELS detector while negative peaks were commonly found using the RI detector. Even though the ELS detector gave significantly higher detector response, the baseline was also much noisier, resulting in no improvement in the sensitivity of the method, as assessed by the LOQ. Over the concentration range investigated, the RI detector had a wider linearity range.

Table 5
Determination of bound HPC in drug colloidal dispersions

	Total HPC concentration in dispersion (mg HPC/g dispersion)		HPC concentration in supernatant (mg HPC/g supernatant)		Percent non-bound HPC		Percent bound HPC	
	ELSD	RI	ELSD	RI	ELSD	RI	ELSD	RI
Sample number								
1	12.63	12.91	10.63	11.35	74.1	77.3	25.9	22.7
2	12.71	12.33	10.99	11.27	76.3	80.7	23.7	19.3
3	12.76	13.68	10.72	11.85	73.7	76.0	26.3	24.0
4	13.34	13.41	11.14	11.72	74.1	77.5	25.9	22.5
Average	12.86	13.08	10.87	11.55	74.6	77.9	25.4	22.1
R.S.D. (%)	2.5	4.5	2.2	2.4	1.6	2.5	4.7	8.9

It is reported in the literature that ELS detection has a wider dynamic range for quantitation and may possibly be used with gradient elution [21,22]; this was not assessed in this study. The ELS detector exhibited better injection precision and method precision, and it also provides the potential for quantitating the active drug. This advantage will be useful if the active drug does not have a UV chromophore. In our case, this application was not made because a separate HPLC method with UV detection was available for the drug analysis.

4. Conclusion

A SEC method and ELS detection conditions for the separation and detection of HPC were optimized to provide maximum peak intensity, adequate peak shape, and minimal baseline noise. The final method was validated for injection precision, linearity, recovery, method precision, and LOQ. The relative merits of ELS and RI detection for this application were compared. The two detectors yielded equivalent mean results for HPC determination; however, ELS detection has better method precision.

A sample preparation procedure was employed for separation of bound and non-bound HPC in a colloidal dispersion formulation. This procedure in combination with the chromatographic quantitation method above was successfully applied to the characterization of HPC surface coverage on drug particles in a representative drug colloidal dispersion. The % bound HPC was determined using both detectors. Results obtained using the ELS detector were significantly more precise.

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References

[1] R.H. Müller, S. Benita, B. Böhm, Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs, Medpharm Scientific, Stuttgart, 1998.
[2] R.H. Müller, C. Jacobs, O. Kayser, Adv. Drug Deliv. Rev. 47 (2001) 3–19.
[3] R.H. Müller, C. Jacobs, Int. J. Pharm. 237 (2002) 151–161.
[4] K.M. Tur, H.-S. Ch'ng, Int. J. Pharm. 160 (1998) 61–74.
[5] I. Henriksen, K.L. Green, J.D. Smart, G. Smistad, J. Karlsen, Int. J. Pharm. 145 (1996) 140–231.
[6] R.H. Müller, R. Becker, B. Kruss, K. Peters, Pharmaceutical Nanosuspensions for Medicament Administration as Systems with Increased Saturation Solubility and Rate of Solution, US Patent No. 5858410.
[7] J. Kevelam, S. Martinucci, J.B.F.N. Engberts, W. Blokzijl, J. van de Pas, H. Blink, P. Versluis, A.J.W.G. Versluis, Langmuir 15 (1999) 4989–5001.
[8] K. Esumi, T. Mizusaki, H. Terayama, Colloids Surf. B 9 (1997) 269–273.
[9] K. Esumi, T. Wake, H. Terayama, Colloids Surf. B 11 (1998) 223–229.
[10] A.H. Kibbe, A. Wade, P.J. Weller (Eds.), Handbook of Pharmaceutical Excipients, second ed., American Pharmaceutical Association, 1998, pp. 223–228.
[11] V.S. Stenkamp, J.C. Berg, Langmuir 13 (1997) 3827–3832.
[12] United States Pharmacopeia, USP26, NF21, Rockville, MD, 2774–2776 (2003).
[13] F.J. Viles, L. Silverman, Anal. Chem. 21 (1949) 950–953.
[14] E.W. Yemm, A.L. Willis, Biochem. J. 57 (1954) 508–512.
[15] L.H. Koehler, Anal. Chem. 24 (1952) 1576–1579.
[16] H.C. Black Jr., Anal. Chem. 23 (1951) 1792–1795.
[17] S.T. Balke, T.H. Mourey, T.C. Schunk, Polym. React. Eng. 7 (1999) 429–452.
[18] G. Delker, C. Chen, R.B. Miller, Chromatographia 41 (1995) 263–266.
[19] R. Madelaine, J.L. Whelan, M.W. Ford, J. Pharm. Biomed. Anal. 30 (2002) 1355–1359.
[20] R.P. Scott, in: E. Katz (Ed.), Handbook of HPLC: Chromatographic Science Series, 78, M. Dekker, New York, 1998, pp. 531–558.
[21] J.M. Charlesworth, Anal. Chem. 50 (1978) 1414–1420.
[22] V.L. Cebolla, L. Membrado, J. Vela, A.C. Ferrando, Semin. Food Anal. 2 (1997) 171–189.
[23] B. Trathnigg, M. Kollroser, B. Maier, D. Berek, M. Janco, Polym. Mater. Sci. Eng. 77 (1997) 48–49.
[24] Y. Wei, M.-Y. Ding, J. Liq. Chromatogr. Related Technol. 25 (2002) 1769–1778.
[25] H. Okumura, N. Kitazawa, S. Wada, J. Oleo Sci. 50 (2001) 249–254.
[26] T.L. Mounts, S.L. Abidi, K.A. Rennick, J. Am. Oil Chem. Soc. 69 (1992) 438–442.
[27] T.A. Foglia, K.C. Jones, J. Liq. Chromatogr. Related Technol. 20 (1997) 1829–1838.
[28] P. Breinhölder, L. Mosca, W. Lindner, J. Chromatogr. B 777 (2002) 67–82.
[29] A. Booth, W. Bowen, R. Reed (Merck Research Lab), manuscript in preparation.
[30] Figure of ELSD courtesy of Polymer Laboratories, Inc.
[31] Polymer Laboratories, Technical Bulletin, TB130, p. 1–3.
[32] G. Saunders, manager of Polymer Characterization Technical Support, Polymer Laboratories, personal communication.
[33] G. Guiochon, A. Moysan, C. Holley, J. Liq. Chromatogr. 11 (1988) 2547–2570.