ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 539–543

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Determination of polyvinyl alcohol in a poly(DL-lactide-co-glycolide) matrix by size exclusion chromatography using evaporative light scattering detection

Kimberly D. Dunn *

Eli Lilly and Company, Lilly Corporate Center, Drop Code 3822, Indianapolis, IN 46285, USA

Received 10 July 2000; accepted 8 November 2000

Abstract

Polyvinyl alcohol (PVA) is used in the manufacture of poly(DL-lactide-co-glycolide) (PLGA) microparticles for the delivery of drugs in an injectable implant form. The levels of residual PVA may affect the release or injectability of the microparticles, and thus must be controlled. Previous work had shown the use of visible detection of iodine–borate complexes of PVA, but this was found to be insensitive and prone to interferences from other formulation components and the sample solvents required. Refractive index detection did not appear to be sensitive enough to detect low levels of PVA. Evaporative light scattering detection was found to be more sensitive and less prone to interferences from the sample matrix than refractive index, and gave reproducible results with acceptable recoveries.

Keywords: Evaporative light scattering detection; Size exclusion chromatography; Polyvinyl alcohol; Microparticles; Injectable implant; Poly(DL-lactide-co-glycolide)

1. Introduction

Aqueous suspensions of polymeric microparticles are often used as an extended release delivery system for intramuscular (IM) depots. The microparticles are prepared using polyvinyl alcohol as an emulsion stabilizer in the solvent extraction method [1]. This process creates the potential for residual PVA that must be monitored for quality and safety purposes. Several other methods of analyses were reported in the literature. Most of these involve the extraction of polyvinyl alcohol from the sample matrix into an aqueous phase, followed by the formation of a PVA–iodine–borate complex that can be detected by visible spectroscopy [2–5]. However, this method suffers from the need to perform an extraction of the aqueous soluble PVA from the organic soluble polymer. For the example presented, the detection of PVA at or below the maximum possible amount of PVA expected of 6% w/w was not possible due to interference from the drug-polymer matrix. In addition, the chloroform extract

* Tel.: +1-317-2766487; fax: +1-317-2775519.
E-mail address: dunn_kimberly@lilly.com (K.D. Dunn).

0731-7085/01/$ - see front matter
PII: S0731-7085(00)00525-2

Table 1
Chromatographic conditions

Column	Tosohaus TSK-Gel GMPW), 30 cm × 7.5 mm (Tosohaus, Montgomeryville, PA)
Flow rate	1 ml/min
Mobile phase	Water containing 0.1% v/v trifluoroacetic acid
Column temperature	37°C
Injection volume	200 μl
Run time	14 min data collection 35 min total run time (to allow for elution of polymer matrix peaks)

exhibited visual particulates that could possibly indicate incomplete dissolution of the microparticles and reduced recovery of PVA.

A method for the analysis of PVA in the polymeric microparticles was developed that involves complete solubilization of the microparticles to release the encapsulated PVA, followed by dissolution of PVA into a hot aqueous solvent. Analysis of the samples was accomplished by size exclusion chromatography, coupled with evaporative light scattering detection. Excellent recoveries were observed in spiked samples with detection limits ranging from 0.8 to 4 μg on column, depending on the style of detector used.

2. Materials and methods

2.1. Materials

PVA (Airvol 205S PVA) was purchased from Air Products and Chemicals Inc (Allentown, PA). All other chemicals were of HPLC grade or better. The PVA standards were prepared from 0.05 to 0.6 mg/ml in 20/80 (v/v) acetonitrile/0.1% v/v aqueous trifluoroacetic acid. These standards were stable for at least 2 weeks when stored at ambient conditions.

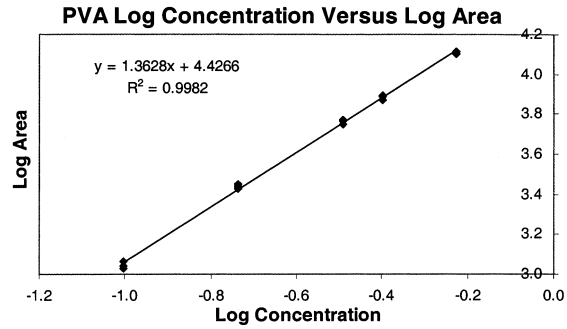

Fig. 1. Typical standard curve of log area versus log concentration.

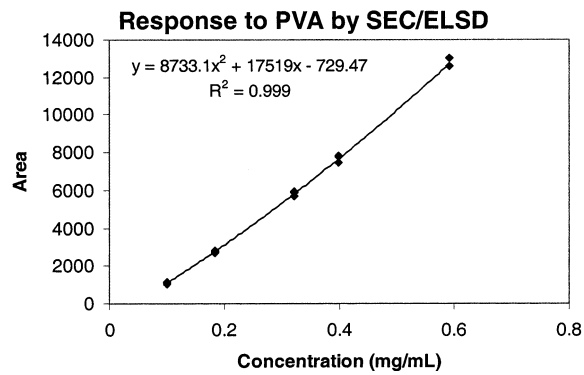

Fig. 2. Typical standard curve of area versus concentration.

Table 2
Detector conditions

Condition	Varex	Alltech	Polymer labs
Drift tube temperature (°C)	167	40	120
Nitrogen flow	64 mm (72 psi)	1.75 SLPM[a]	1.0 SLPM
Low temperature adapter temperature (°C)	Not applicable	40	Not applicable
Nebulizer temperature (°C)	Not applicable	Not applicable	85

[a] SLPM, standard liters per minute.

2.2. Equipment

SEC analyses were performed on an isocratic HPLC system. Due to the high molecular weight of the PVA of approximately 50 000, an SEC analysis procedure was chosen. The PVA used for quantitation was a sample of the same material used in manufacturing of the microparticles. An evaporative light scattering detector (ELSD) was used for the initial method development (Varex ELSD IIA, Deerfield, Illinois). Further evaluations were performed on an ELSD with a low temperature adapter (LTA) (Alltech 500 ELSD with LTA, Deerfield, IL) and a Polymer Laboratories ELSD (Model PL-ELS 100, Amherst, MA). Similar results were obtained, but the latter detectors were approximately $2\frac{1}{2}$–5 times more sensitive than the former. The chromatographic conditions are shown in Table 1. The conditions for each of the three different detectors examined are shown in Table 2.

Table 3
Recovery of PVA from PLGA matrix

Sample no.	Weight polyvinyl alcohol added (mg)	Recovery
1 (65:35)	0.578	97.74
2	0.569	98.66
3	0.607	101.22
Average		99.21
% RSD		1.82
4	1.347	96.03
5	1.341	99.42
6	1.309	99.22
Average		98.22
% RSD		1.94
7	2.131	100.72
8	2.220	99.81
9	1.931	98.90
Average		99.81
% RSD		0.91
1 (75:25)	0.610	99.85
2	0.557	100.26
3	0.597	100.32
Average		100.14
% RSD		0.25
4	1.303	98.64
5	1.226	97.39
6	1.280	99.91
Average		98.65
% RSD		1.28
7	2.248	99.21
8	2.021	100.39
9	1.947	99.84
Average		99.81
% RSD		0.59
Overall average		99.31
Overall % RSD		1.29

2.3. Sample preparation

Samples were prepared by dissolving approximately 50 mg of PLGA microparticles in 1 ml of acetonitrile in a 5-ml volumetric flask. After vortexing and brief sonication in a bath, 0.1% v/v aqueous trifluoroacetic acid at approximately 90–95°C was added, and the sample vortexed briefly prior to 20 min of sonication. The sample was then cooled, and filled to volume with 0.1% v/v aqueous trifluoroacetic acid. Finally, the sample was filtered through 0.45 μm PTFE filters and analyzed. The samples are stable for 2 days at room temperature. Spiked sample preparation was accomplished by adding dry PVA (Airvol 205S PVA, Air Products and Chemicals) to the dry microparticles prior to sample preparation. Since this method does not address any entrained PVA, a separate experiment was conducted to prove that the PVA could be removed from the microparticles using this method. A solution of 6% w/v PVA in water was prepared and approximately 1 g of microparticles was added to the solution and swirled well. The solution was then filtered and the microparticles collected and allowed to dry overnight. The dry microparticles were then weighed and prepared as above. Further proof that the method was capable of detecting PVA in the formulation was obtained at a later date, when samples containing detectable PVA were prepared. These samples had levels of PVA on the order of 0.1–0.3% w/w.

3. Results and discussion

The limits of detection for PVA ranged from 0.8 to 4 μg on column depending on the detector

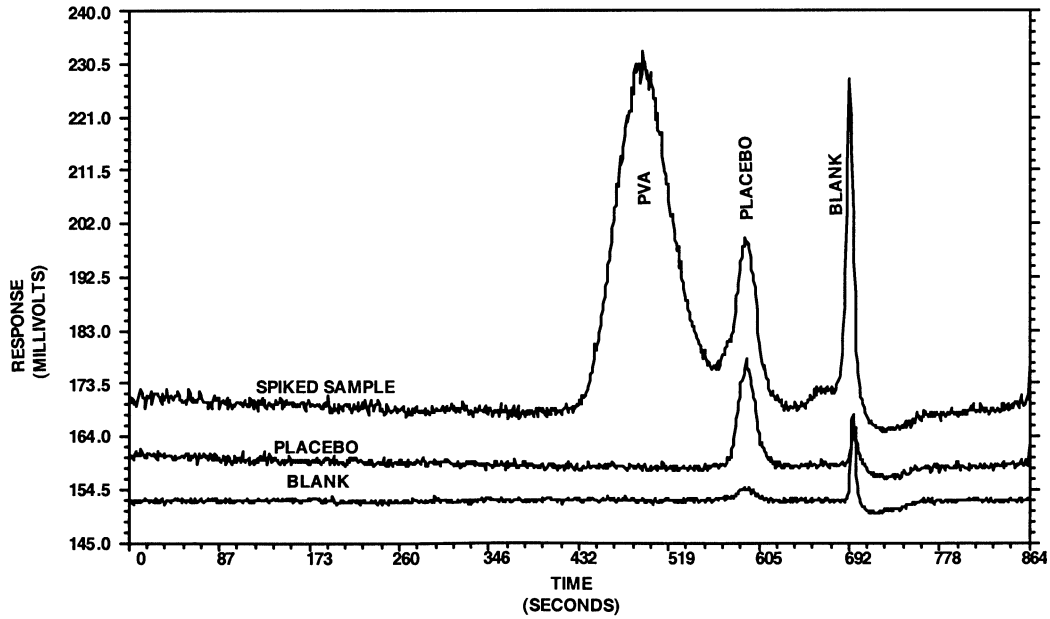

Fig. 3. Representative chromatograms blank, placebo and sample solutions.

used. The Alltech and Polymer Laboratories detectors gave approximately 3 and 5 times lower detection limits for an aqueous system, respectively. For both models of detector, the method showed a good R^2 value when the logarithm of the area is plotted versus the logarithm of the concentration of PVA. Typically, ELSD is nonlinear over this wide of a concentration range, and the results must be treated as a logarithm to obtain a linear curve [6]. Example standard curves both prior to and after logarithmic treatment are shown in Fig. 1 and Fig. 2.

Recovery of PVA from the sample matrix was determined by weighing solid PVA with solid microparticle samples and preparing them as discussed under sample preparations. Spiked and unspiked samples of two different polymer ratios (65:35 and 75:25) were analyzed in triplicate at three different levels. The polymer ratios had no affect on the recovery observed as shown in Table 3. Fig. 3 shows that no interfering peaks from the sample matrix or the solvents were observed. The two peaks observed in the placebo solution are likely due to the acetonitrile used in the sample solvent and the excipients in the placebo formulation, although these peaks have not been specifically identified. The sample prepared by placing microparticles in an aqueous PVA solution demonstrated that PVA could be easily observed, and that the extraction method works well for these samples. Analyses of multiple batches of these microparticles have shown that different levels of PVA other than the spiked samples can be observed.

4. Conclusions

A simple chromatographic method for the analysis of PVA in a polymer matrix has been developed. The advantages of this method are that low concentrations of PVA can be detected with minimal interferences. The method is simple and can be applied to a wide variety of polymer matrices that are insoluble in the sample solvent. It also allows samples to be analyzed that are not compatible with currently published methods that require extraction and spectroscopic detection.

References

[1] T.R. Tice, R.M. Gilley, Microencapsulation process and products therefrom, US Patent Number 5 507 609.
[2] J.G. Pritchard, D.A. Akintola, Complexation of polyvinyl alcohol with iodine, Talanta 19 (7) (1971) 877–888.
[3] D.P. Joshi, Y.L. Lan-Chun-Fung, J.G. Pritchard, Determination of poly(vinyl alcohol) via its complex with boric acid and iodine, Anal. Chim. Acta 104 (1) (1979) 153–160.
[4] J. Urbanski, Analysis of poly(vinyl alcohol) and its derivatives, in: Handbook of Analysis of Synthetic Polymers, Halstedt Press, New York, 1977, pp. 391–395.
[5] M. Singh, X. Li, H. Qiu, T. Zamb, C.Y. Wang, D. O'Hagan, Residual polyvinyl alcohol content in polylactide-co-glyocolide microparticles prepared by solvent evaporation method, Proc. Int. Symp. Control Rel. Bioact. Mater. 23 (1996) 367–368.
[6] L.E. Oppenheimer, T.H. Mourey, Examination of the concentration response of evaporative light scattering/mass detection, J. Chromatogr. 323 (1985) 297–304.