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In situ Fiber Optic Dissolution Monitoring of a Vitamin B_{12} Solid Dosage Formulation

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Abstract

The rapid dissolution rate of many water-soluble, immediate release solid dosage forms requires a dissolution analyzer that is fast, sensitive, customizable for frequent measurements, and able to generate data of high precision and quality. These requirements fit perfectly with today's *in situ* fiber optic technology. The Rainbow Dynamic Dissolution Monitor was used in this study to demonstrate and evaluate the dissolution behavior of 500 mg Vitamin B_{12} (cyanocobalamin) tablets in degassed water, simulated gastric fluid (SGF), and phosphate buffer at stirring speeds as high as 100 rpm. Upon dissolution, Vitamin B_{12} tablets produce rather turbid suspensions that may be expected to cause problems for *in situ* measurements. However, the effect of light scattering by suspensions is largely eliminated when the second derivative of the absorbance signal is used in quantitation. Excellent data correlation was observed between the different media and the standard when using this method.

Introduction

Vitamin B_{12}, or cyanocobalamin (Fig. 1) promotes normal growth and maturation of human cells and maintains the myelin sheath in the nervous system. Deficiency of this vitamin typically generates reproductive complications, neuropathy, dermatitis, and pernicious anemia in humans.

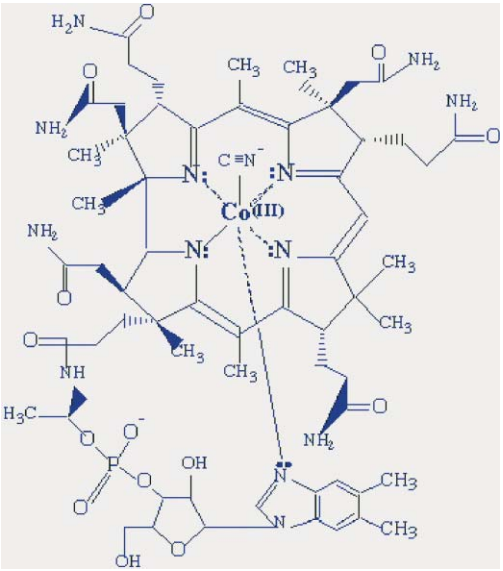

Figure 1: Structure of cyanocobalamin (Vitamin B12)

The ideal diet supplies adequate quantities of all B vitamins, including thiamine (B_1), pantothenic acid (B_3), riboflavin (B_2), and pyridoxine (B_6). Increased demand of a single vitamin may be assisted through nutritional supplementation. However, a deficiency of one member of this group is often coupled with deficiencies of one or more of the others. While single solid dosage formulations containing a single B vitamin are available commercially, treatment with a B-complex regimen is often recommended [1].

Although Vitamin B_{12} was the last vitamin to be discovered, its structure and properties have been studied extensively since its isolation in 1948. Synthesized in nature only by microorganisms, the vitamin's cobalt atom may bond with a hydroxyl group, as observed in samples isolated from the liver (hydroxycobalamin) or may exist as nitrocobalamin, which has been isolated from microorganisms. The laboratory synthesis of Vitamin B_{12} results in the attachment of a cyanide ligand to the central cobalt atom. This form is typically employed in the supplementation of foods and in tablets. Cyanocobalamin is soluble in various solvents and displays its greatest stability in the pH range 4.5-5.0.

Dissolution profiles of this highly potent water-soluble vitamin reaches completion in as little as 3 minutes, warranting a monitoring system that is highly sensitive, efficient, and fast enough to produce a well defined dissolution profile. Besides meeting all of these criteria, *in situ* fiber optic UV dissolution measurement offers clear advantages over HPLC through its preservation of media volume, reduced waste disposal, low labor intensity, potential for absence of moving parts, and lack of elaborate sipping mechanisms complicating compliance with 21 CFR Part 11. Additionally, the *in situ* fiber optic probe systems allow dissolution monitoring of UV-responsive compounds using a real-time display of the dissolution profile and significantly reducing the turn-around time for measurement and report generation when compared to HPLC assays [2].

Dissolution of 500-mg Vitamin B_{12} tablets produces a slurry of excipient particles that can clog and damage the plumbing and filters of HPLC-assisted monitoring systems. For unfiltered *in situ*, UV-based systems, the high turbidity of the

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vessel suspension produces Tyndall scattering causing the UV spectrum of an analyte to exhibit a sloping baseline. Simple baseline subtraction algorithms of conventional spectroscopy analysis software cannot eliminate such a baseline.

Bynum et al. demonstrated that this spectral slope can be removed through proper second derivative treatment of the measured data.

The second derivative of the absorbance spectrum for a turbid solution generates a quantifiable spectrum identical to the second derivative of a spectrum collected for a standard solution containing only the active ingredient at the same concentration [2]. *SmartWare*, the software package accompanying the Rainbow Dynamic Dissolution Monitor, allows development of methods using both baseline subtraction and second derivative treatment in the data analysis.

This paper demonstrates a quantification method for the dissolution of immediate release Vitamin B_{12} tablets using UV fiber optic dip probes connected to the 6-channel Rainbow Dynamic Dissolution Monitor. To detect possible differences in dissolution behavior, this solid dosage form product was evaluated in three common degassed media: deionized water, simulated gastric fluid (without enzymes), and phosphate buffer at pH 7.4. The dissolution profiles for each of the three media were analyzed in replicates of six using the second derivative treatment of the sample spectra.

Materials & Methods

Simulated Gastric Fluid: SGF (without enzymes) was prepared using the protocol in USP 25 NF 20; 12.0 g of sodium chloride (SIGMA ACS Reagent Lot# 082K0242) was added to a volumetric flask containing 42.0 mL 12 M HCl (SIGMA-Aldrich ACS Reagent Batch # 05013TA) and diluted with deionized water to a total solution mass of 5995 g. The mixed solution was then heated to 40° C and degassed by sonication.

Phosphate Buffer, pH 7.4: The phosphate buffer was prepared by adding 15.7 g anhydrous KH_2PO_4 (SIGMA Lot# 42K0202) to 2.8 g NaOH (Min. 98% SIGMA Lot# 092K0132). The solution was then diluted with deionized water to a mass of 5926 g, mixed, heated to 40° C, and degassed by sonication.

Vitamin B_{12} Standard Solution: Weighing 55.6 mg Vitamin B_{12} into a flask and diluting to 2000 mL produced a standard solution of Vitamin B_{12} (SIGMA Vitamin B_{12} Lot# B41949). One 5-mL aliquot from this standard solution was transferred to a 250-mL volumetric flask and diluted to volume with deionized water preheated to 37 °C. The B_{12} standard to be used for the SGF assay was prepared by adding 1750 mL 12 M HCl and 0.5 g NaCl prior to dilution. The B_{12} standard used for the phosphate buffer was prepared by adding 0.11 g 98% (Min) NaOH and 0.65 g KH_2PO_4 prior to final dilution. The temperature of all standard solutions used in the experiments was allowed to equilibrate to 37° C in each case.

Vitamin B_{12} Sample Tablets: Nature Made brand 500-mg solid vitamin B_{12} tablets (Lot# MC10095, Exp. 02/06) were purchased for use in this dissolution study. Placebos were not available.

The Rainbow Dynamic Dissolution Monitor (Delphian Technology Inc., Woburn, MA, www.delphian-tech.com) contains a Cathodeon Type J75 Deuterium (D_2) lamp that transmits its signal via a furcation cable to supply the primary signal to six stainless steel probes; each probe is positioned in a dissolution vessel containing the liquid medium. As shown in Fig. 2, a removable tip accompanies the probe. Each tip, the length of which defines the spectroscopic path length, contains a mirror to reflect the signal from the analyte molecule into the probe's receiving channel (see Fig. 3) and back to its own Zeiss MMS-UV photo diode array (PDA) spectrometer covering

Figure 2: Fiber optic dip probe showing removable tip (20 mm path length)

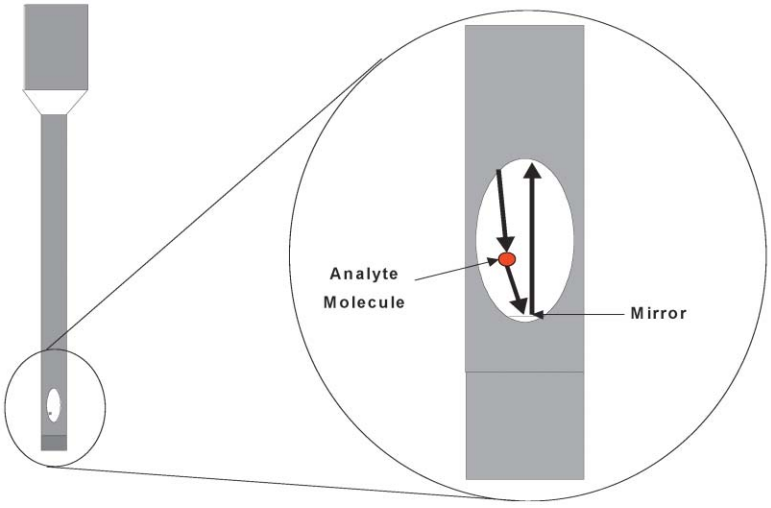

Figure 3: Transmission of UV signal from the analyte via the fiber optic probe

In situ Fiber Optic Dissolution Monitoring … *continued*

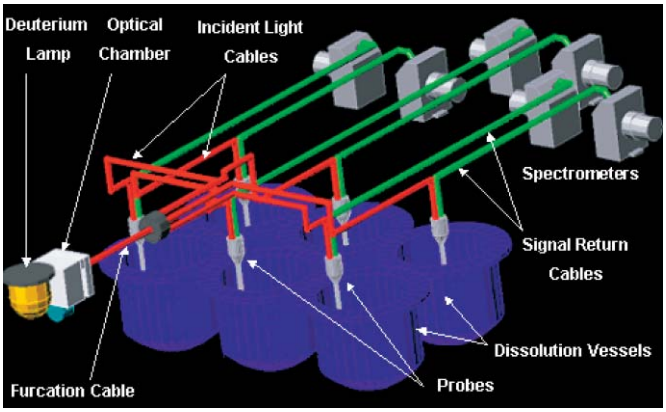

Figure 4: Schematic diagram of the Rainbow installed on a dissolution bath

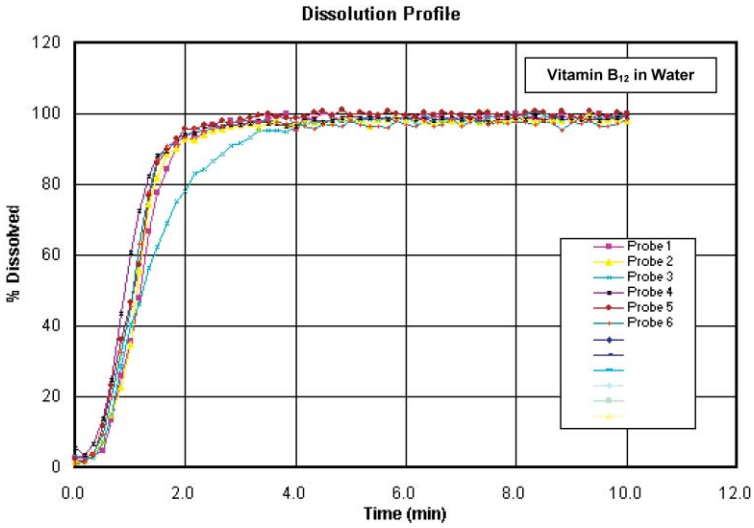

Figure 5: Dissolution profiles of Vitamin B_{12} in water

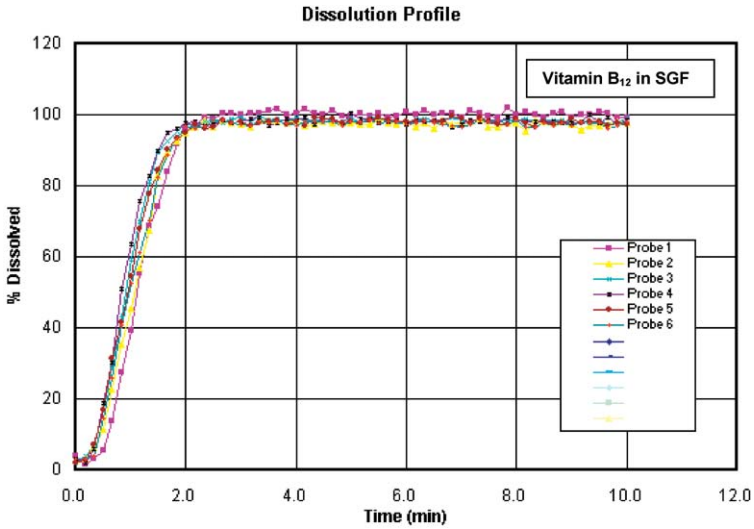

Figure 6: Dissolution profiles of Vitamin B_{12} in SGF

Table 1: Vitamin B12 dissolution data for three media

Dissolution Medium	Second Derivative		Baseline Subtraction	
	% Dissolved after 10 min.	% RSD	% Dissolved after 10 min.	% RSD
Water	99.5	1.5	101.6	1.2
SGF	98.4	0.9	100.4	1.4
Phosphate buffer (pH 7.4)	95.1	0.9	100.4	3.2

the wavelength range of 230-380 nm. The 20-mm path length seen in Fig. 2 was used in all experiments.

Fig. 4 shows a schematic layout for the complete apparatus. A *VanKel* model VK 7010 dissolution bath was heated to 37°C and fitted with USP Apparatus 2 (paddles) and configured to rotate at 100 rpm. This stirring speed is higher than the 50 to 75 rpm recommendation in the FDA guidance on immediate release products and was chosen to evaluate the ability of the dissolution monitor to keep up with the dissolution process even beyond the recommended speed.

Due to the photosensitivity of cyanocobalamin, the bath was shielded to prevent exposure to ambient light. Six vessels, each containing 900 mL dissolution medium, were then placed in the bath and allowed to achieve thermal equilibrium with the paddles and probes immersed. After normalization of probe energy input to the photometers and collection of the dark, 100% transmittance, and (excipient free) standard solution spectra, a tablet was delivered to each of the six vessels followed by immediate activation of paddle rotation. Monitoring the absorbance maximum at 362 nm for cyanocobalamin, and using the second derivative of the spectra in the 355 to 368 nm range for quantitation, *SmartWare* traced the real-time progression of the dissolution profile and subsequently generated the statistical data from the completed assay. Dissolution data were collected and plotted as %-dissolved every 10 seconds during the 10-minute run.

Results

Table 1 summarizes the data collected for the dissolution experiments in the three different media and represents averages of six tablets per medium. It compares the data originally obtained with results recalculated using simple baseline subtraction. Since there is no truly flat portion on the spectrum, an ideal wavelength for baseline subtraction is not available. A high wavelength of 382 nm was chosen for the compensation because Tyndall scattering occurs at significantly shorter wavelengths.

The tested Vitamin B_{12} product shows recovery of the active ingredient within 5% in all three media with RSD values

*See **In situ** Fiber Optic Dissolution … continued on page 24*

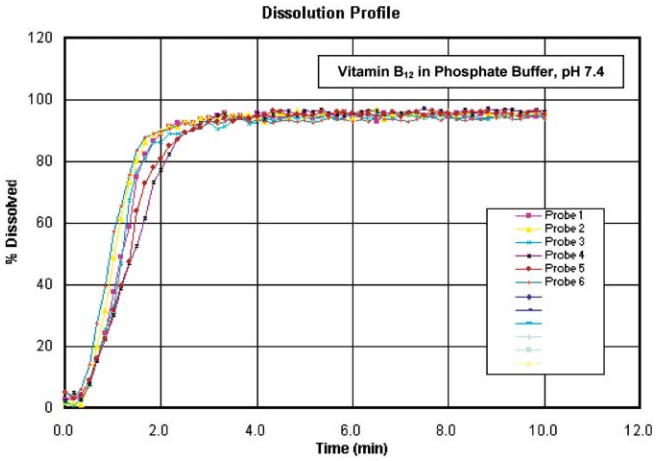

Figure 7: Dissolution profiles of Vitamin B_{12} in phosphate buffer

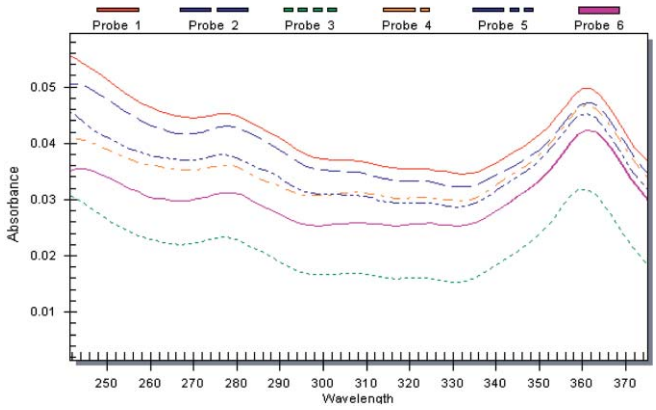

Figure 8: UV spectrum of Vitamin B12 for all six probes at the completion of the experiment

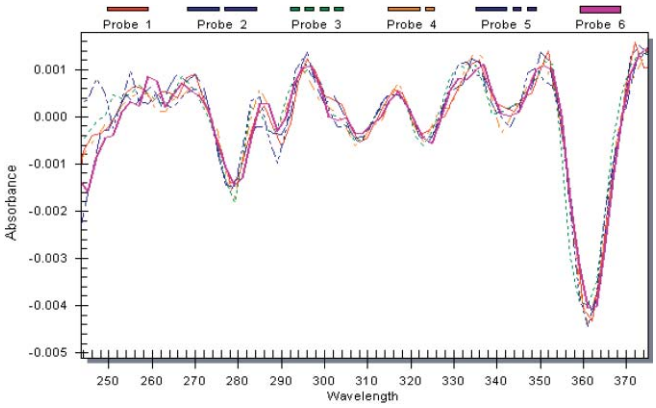

Figure 9: Second derivative of Vitamin B12 UV spectrum for all six probes at the completion of the experiment

of less than 2% for the individual medium when the second derivative is used. The results are not much different when a simple baseline subtraction is used, although the %RSD values are higher.

Fig. 5 – Fig. 7 indicate that this highly potent vitamin product is almost completely dissolved 3 minutes from the start of the experiment, regardless of medium composition.

The lower slope of the tablet monitored by probe 3 in water (Fig. 5) reveals a slightly slower rate of dissolution of the tablet in vessel 3.

Fig. 8 shows the UV spectrum for all six probes at the completion of the experiment in water. The sloping baseline observed in each spectrum is characteristic of a tablet generating high turbidity during dissolution. In the case of Vitamin B_{12}, a naturally sloping spectrum over the entire wavelength range covered by the Rainbow's spectrometer adds to the slope and offset created by the excipient.

Because spectra of this type prevent selection of a really satisfactory wavelength for baseline correction by subtraction, the second derivative treatment was applied to the entire spectrum for each of the six channels. As seen in Fig. 9, the second derivative spectra feature a prominent negative peak at 362 nm, the wavelength of the positive peak of the basic UV spectrum. The uniformity of the second derivative spectra from the six channels is noteworthy, too.

To use the negative peak for quantitation, a range approximately centered on the peak is chosen and an area-under-the-curve integration is applied to reduce some of the uncertainty (noise) introduced by the second derivative treatment of the UV spectrum. The choice of the width of the range is not critical and is best made by inspecting the waveform during method development. *SmartWare* allows this range to be changed at will even after the spectra have been collected. For compliance reasons, the report will indicate that a change was made and display the original wavelength settings as well. In this case, a range of 355-368 nm was used. The second derivative and subsequent AUC calculation may be done in real time and thus allows real time display of the dissolution curve as the dissolution progresses.

Conclusion

The Rainbow Dynamic Dissolution Monitor offers the advantage of very high data density when monitoring in real-time the dissolution profile of immediate release solid dose formulations. It is useful for highly water-soluble, rapidly dissolving products containing UV chromophores, such as Vitamin B_{12} tablets. For this product, there is no significant difference between the dissolution profiles in the three media tested. Well-defined dissolution profiles are obtained in all cases with data collected every 10 seconds. The inherent chal-

lenge to *in situ* measurements is the obvious lack of filtration before measurement. However, second derivative treatment of the UV spectrum makes it possible to remove, without significant loss in sensitivity and repeatability, the effects of the sloping baseline often encountered in spectra of highly turbid samples.

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