International Journal of Pharmaceutics, 50 (1989) 39–43
Elsevier

IJP 01678

The investigation of dual-wavelength spectroscopy for the analysis of dissolved drug in microcapsule suspensions

Merrick L. Shively [1] and Anthony P. Simonelli [2]

[1] *The School of Pharmacy, University of Colorado, Boulder, CO 80309 (U.S.A.)*
and [2] *The School of Pharmacy, The University of Connecticut, Storrs, CT 06268 (U.S.A.)*

(Received 11 March 1987)
(Modified versions received 25 November 1987 and 11 April 1988)
(Accepted 18 July 1988)

Key words: Dual-wavelength; Ultra-violet spectroscopy; Suspension; Microcapsule; Turbid solution

Summary

The rationale for using the method of dual-wavelength spectroscopy to study suspensions and other highly turbid systems was theoretically developed. In order to demonstrate the usefulness of this technique, dual-wavelength spectroscopy was used to accurately determine the release of hydrocortisone 21-acetate from microcapsules in aqueous suspension. In contrast to other analytical procedures, experimental artifacts introduced by filtering and the tableting of microcapsules were eliminated. In addition dual-wavelength spectroscopy enabled extremely low concentrations, i.e. 0.6 μg/ml hydrocortisone 21-acetate, to be reproducibly determined, i.e. $\pm$ 2% (n = 3).

Introduction

The release of drug from microcapsules is presently studied by two basic methods, i.e. as suspensions (Brophy and Deasy, 1981; Iso et al., 1985) or tablets (Higuchi et al., 1967). Basically, the first method consists of filtering a suspension to produce a filtrate for later analysis. The second method to study the dissolution of powders consists of compressing the powder into a tablet die to produce a constant surface area and a clear solution.

Each of the previously described analytical techniques introduces experimental uncertainty. The experimental uncertainty from the suspension method is due to the filtering of microcapsules from the release media. The continual filtering of microcapsules from the release medium reduces the area available for diffusion in a random fashion. The dependence of the surface area for the diffusion of drug from microcapsules has been thoroughly described (Crank, 1975). In addition, adsorption of drug to the filter can introduce significant errors, particularly at low drug concentrations.

Due to fractured microcapsules produced by compressional forces present during the formation of a microcapsule tablet, experimental artifacts may be introduced. Diffusion, chemical stability, as well as other properties may be affected by the presence of ruptured microcapsules. In fact, the ability of pressure to rupture gelatin microcaps-

Correspondence: M.L. Shively, The School of Pharmacy, University of Colorado, Boulder, CO 80309, U.S.A.

ules containing dyes is a marketable property of microcapsules, i.e. carbon-less carbon paper (Green, 1956).

A review of the above analytical procedures and their inherent disadvantages prompted these authors to investigate alternative analytical techniques. The most promising analytical technique for the purpose of suspension analysis was dual-wavelength spectroscopy. Cowles (1965) through his studies of enzyme kinetics, demonstrated that dual-wavelength spectroscopy was useful for the measurement of small absorbance changes in systems containing high absorbing backgrounds. The technique consists of using a reference wavelength to correct for the highly absorbing background. Since the scattering of light by suspended particles produces an apparent high absorbance, the ability of dual-wavelength spectroscopy to correct or subtract the effect of a highly absorbing background was felt to be particularly advantageous for the analysis of microcapsule suspensions.

Therefore, this investigation was initiated in order to highlight the applicability of dual-wavelength spectroscopy and to quantify the release of drug from microcapsule suspensions. In addition, dual-wavelength spectroscopy requires extremely small quantities of drug for analysis. The ability to analyze small quantities is especially advantageous in an era where the use of bioengineered compounds (peptides, proteins, growth factors, and hormones) is steadily increasing. The technique of dual-wavelength spectroscopy for use in the analysis of microcapsule suspensions is devoid of intrinsic uncertainties introduced by filtering (i.e. adsorption of drug and reduction of surface area), and tableting methods (rupture of microcapsules).

Materials and Methods

Materials

The macromolecule used to produce microcapsules was a biodegradable polymer developed by Bitritto et al. (Bitritto, 1979). The polymer was prepared by the melt polymerization of a diacid, tartaric acid (Aldrich Chemical Company, reagent grade) and a long-chain diol, 1,10-decanediol (Aldrich Chemical Company, reagent grade). After several washings with ether the resulting polymer was a fine (20–80 μm), white, semi-crystalline powder which had a molecular weight of approximately 925 g/mol and a melting point of 82 °C.

The microcapsule core material was hydrocortisone 21-acetate (HCAC), (Sigma Chemical Co.). The solubility of HCAC was determined to be 0.01 mg/ml in double distilled water at 25 °C.

The release apparatus was a 500 ml jacketed beaker which was thermostatically controlled at 25 °C. A constant stirring rate of 400 rpm was provided by a synchronous motor (Model CA, Hurst Manufacturing Corp., Princeton, IN). For extremely small samples, dissolution may be directly carried out in a thermostatically controlled and stirred cuvette.

Microencapsulation procedure

Coacervation of the bio-degradable polymer was used to produce microcapsules. The coacervation system consisted of the polymer, solvent (ethanol), and a non-solvent (water). Microcapsules were produced by titrating non-solvent (water) into a 1 mg% solution of polymer in ethanol, in which hydrocortisone 21-acetate has been suspended, at a rate of 33 ml/h. The polymer to drug or polymer to core ratio (PCR) was one to two (1 : 2). The entire encapsulation of hydrocortisone 21-acetate was carried out in a thermostatically controlled beaker at 25 °C and stirred at 100 rpm (Shively, 1986). The resulting microcapsules (10–50 μm as determined by scanning electron microscopy) were filtered, dried, and stored under vacuum.

Release of drug from microcapsule powder

Double distilled water (500.0 ml) was added to the release apparatus (Simonelli, 1969) and allowed to equilibrate to 25 °C. Hydrocortisone 21-acetate microcapsules (1.5 mg) were added to the release media. Samples of the release media were removed as a function of time and assayed. In order to maintain a closed system (in terms of surface area) the samples were returned to the release apparatus after analysis. The cuvette was rinsed several times with the release media between assaying intervals.

Sedimentation studies

Solutions of hydrocortisone 21-acetate (0.05, 0.3, and 0.6 μg/ml) were prepared using double-distilled water. These concentrations were specifically chosen to bracket the concentrations which were achieved in preliminary microcapsule release studies, (less than 10% HCAC solubility). 1.0 ml of solution was added to a quartz cuvette with a 1-cm path length. 1.0 mg of a highly absorbing but insoluble suspendable material (decolorizing carbon, J.T. Baker) was added to the cuvette. The absorbance of the sample, postadsorption equilibrium, was then collected while sedimentation was occurring as a function of time.

Quantitative analysis

The monitored sample wavelength for hydrocortisone 21-acetate (HCAC) was determined to be 247.6 nanometers using a dual-wavelength spectrophotometer (DW2, SLM/Aminco, Urbana, IL) in the conventional split-beam configuration. The dual-wavelength mode was then utilized to empirically define the reference wavelength for HCAC as 277.0 nm. The empirically determined reference wavelength may be less than or greater than the sample wavelength but must be a wavelength at which the compound of interest has no absorption. The sample is then added to the sample cuvette and the resulting absorbance monitored as a function of time.

Theoretical Considerations

The usefulness of dual-wavelength spectroscopy may be more fully understood by mathematically describing the progression of data, in the form of electrical signals generated by the photomultiplier tube (PM), through the spectrometer. The mathematical relationships to describe these functions were not currently accessible in the pharmaceutical literature at the time these studies were initiated and are therefore presented at this time.

The voltage output from the PM is a function of the absorbance of the dissolved species and the scattering of light by suspended or undissolved particles. The necessary relationships were derived by analyzing the voltage outputs from the photomultiplier and the ratio logarithmic converter (Cowles, 1965). The modulated signal generated by a photomultiplier at the sample wavelength (V_1) may be described as follows:

$$V_1 = V_\alpha \, e^{-(A_1+\tau_1)} \tag{1}$$

where V_α = output without sample, A_1 = absorbance at wavelength 1, τ_1 = turbidity at wavelength 1.

The modulated output signal generated by the PM for the second or reference wavelength (V_2) can be described by Eqn. 2.

$$V_2 = V_\beta \, e^{-(A_2+\tau_2)} \tag{2}$$

where V_β = output without sample, A_2 = absorbance at reference wavelength, τ_2 = turbidity at reference wavelength.

Dual-wavelength spectroscopy differs from conventional methods of ultra-violet or visible spectroscopy by the incorporation after the photomultiplier of a ratio logarithmic converter as opposed to a conventional ratio type converter. The modulated signals from the photomultiplier are passed through the ratio logarithmic converter whose input-output function is described as:

$$V_{o_i} = V_b \text{nlog}(V_i/V_a) \tag{3}$$

where V_a = reference level of converter, V_b = sensitivity factor (volts/decade), V_i = input voltage, e.g. V_1 or V_2, V_{o_i} = output voltage for wavelength i. The voltage output of the sample and reference wavelengths as it passes from the ratio logarithmic converter may be described by substituting Eqns. 1 and 2 into Eqn. 3. The resulting relationships are given by Eqns. 4 and 5.

$$V_{o_1} = V_b \text{nlog}(V_\alpha/V_a) - V_b(A_1 + \tau_1) \tag{4}$$

$$V_{o_2} = V_b \text{nlog}(V_\beta/V_a) - V_b(A_2 + \tau_2) \tag{5}$$

Conventionally, the observed or recorded output is simply the voltage difference between the input from the sample wavelength and the input from the reference (analogous to Eqn. 6a). For cases when a ratio logarithmic converter is utilized

the input functions are more complex, Eqns. 4 and 5. The expressions for the voltage difference are therefore given by Eqns. 6a and 6b.

$$\Delta V = V_{o_1} - V_{o_2} \tag{6a}$$

$$\Delta V = V_b \text{nlog}(V_\alpha / V_\beta) - V_b(A_1 - A_2 + \tau_1 - \tau_2) \tag{6b}$$

As part of the initial calibration process, the converter response is adjusted so that V_β is equal to V_α. Experimentally, V_α is predetermined by the sample wavelength but V_β may be varied by adjusting the incident intensity of the reference wavelength. As a result of the V_α/V_β ratio being equal to 1, the resultant voltage difference (Eqn. 6a or 6b) is simplified (Eqn. 7).

$$\Delta V = -V_b(A_1 - A_2 + \tau_1 - \tau_2) \tag{7}$$

Since no assumptions or approximations were made in Eqn. 7, there are no theoretical restrictions on the magnitude of the absorbance or turbidity differences which may be detected and linearly recorded. This enables extremely large as well as small changes in absorbance and turbidity to be linearized and recorded. The enhanced sensitivity of dual-wavelength spectroscopy is due to its electronic design rather than to some increase in the drug's molar absorptivity. As with conventional methods of UV/Vis spectroscopy, the net absorbance is calibrated with concentration using Beer's law. An internal rotating chopper apparatus is used to generate the background or scattering corrected absorbance at a rate of 1000 corrections per min. This is not the case for conventional UV/Vis spectroscopy (including diode array methods) where it is mandatory, if the results are to be linearized, that the background absorbance be small and the total net absorbance be equal or less than one absorbance unit.

Results and Discussion

In order to verify this method of analysis, decolorizing carbon was added to three unstirred hydrocortisone 21-acetate (HCAC) solutions and the respective absorbances were recorded as a function of time. Note that the spectrometer can not differentiate between light scattered from suspended HCAC, charcoal, or microcapsules. The results of these sedimentation studies are shown in Fig. 1. Analysis of Fig. 1 shows that the absorbance of HCAC was not affected, other than from adsorption, by the presence of suspended particles or particle sedimentation. For example, for all cases at time zero, the system was highly turbid while at the completion of the experiment there was little or no suspended material present.

The release of HCAC from microcapsules was recorded (Fig. 2). From the analysis of the release profile (Fig. 2) it is evident that two phases exist within the profile, i.e. an initial linear segment and a longer curving portion. The initial linear segment may be rationalized as that portion of the profile in which a constant concentration or potential gradient (activity equal to one within the microcapsule) is found. The point at which the release profile begins to curve (approximately 25 min) signifies the probable depletion of solid drug within the microcapsules (activity less than one) and a resulting continuous decrease in the concentration gradient and diffusion potential as a function of time. The estimate of the standard error for 3 trials was calculated to be 1.95%.

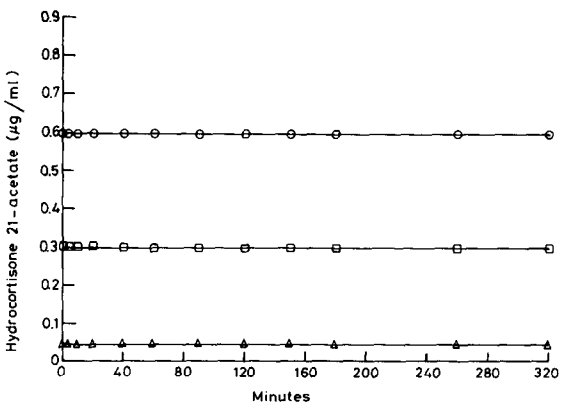

Fig. 1. Concentration of hydrocortisone 21-acetate during sedimentation of decolorizing carbon in distilled water (25 °C). ▲, 0.05 μg/ml hydrocortisone; □, 0.3 μg/ml hydrocortisone; ○, 0.6 μg/ml hydrocortisone.

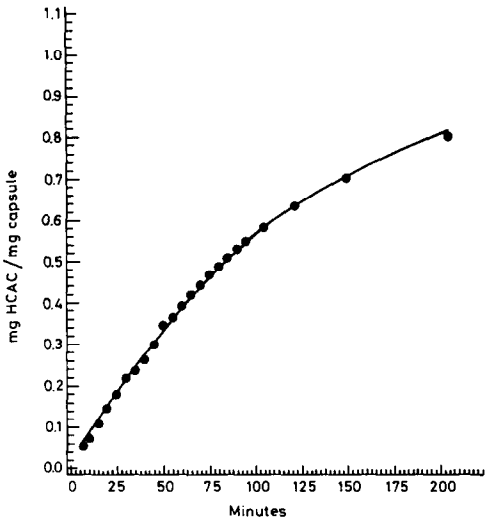

Fig. 2. Release of hydrocortisone 21-acetate from microcapsules as a function of time in distilled water (25 ° C).

It is important to note that the concentration of hydrocortisone 21-acetate which would have been required if conventional split beam spectroscopy (including diode array detectors) were used would be significantly greater than 10% of the solubility of HCAC producing non-ideal or non-sink conditions. Had the concentration of HCAC been greater than 10% of HCAC's solubility, any curvature in the release profile could then be explained as an effect of non-sink diffusion conditions.

The authors acknowledge that several alternatives to the method of dual-wavelength spectroscopy for the analysis of microcapsule suspensions exist (i.e. experimentation with filter media, throughput volumes, HPLC assays, etc.), but these methods tend to be costly and time-consuming. The ultimate potential of dual-wavelength spectroscopy lies in its versatility and freedom from common sources of uncertainty.

Acknowledgment

This work was supported in part by a fellowship from Richardson-Vicks Inc., Wilton, Connecticut as well as by a predoctoral fellowship from The University of Connecticut, Storrs, Connecticut.

References

Bitritto, M.M., Bell, J.P., Brencle, G.M., Huang, S.J. and Knox, J.R., Synthesis and biodegradation of polymers derived from alpha-hydroxy acids, *J. Appl. Polymer Sci.*, 35 (1979) 405–414.

Brophy, M.R. and Deasy, P.B., Influence of coating and core modifications on the invitro release of methylene blue from ethylcellulose microcapsules produced by pan coating procedure, *J. Pharm. Pharmacol.*, 33 (1981) 495–499.

Cowles, J.C., Theory of dual-wavelength spectrophotometry for turbid samples, *J. Opt. Soc. Am.*, 55 (1965) 690–693.

Crank, J., "*The Mathematics of Diffusion*", 2nd edn., Clarendon, Oxford, 1975, pp. 89.

Green, B.K. and Schleicher, L. *U.S. Patent 2,730,456*, January 10, 1956.

Higuchi, W.I., Bernardo, P.D. and Mehta, S.C., Polymorphism and drug availability II: dissolution rate behavior of the polymorphic forms of sulfathiazole and methylprednisolone, *J. Pharm. Sci.*, 56 (1967) 200–207.

Iso, M., Kando, T. and Omi, S., A fundamental study of the microencapsulation procedure utilizing coacervation in a polystyrene-cyclohexane solution, *J. Microencapsulation*, 2 (1985) 275–287.

Shively, M.L., *The study of Surface Structure in Biodegradable Microcapsules*, Dissertation, University of Connecticut, Storrs, CO, 1986.

Simonelli, A.P., Mehta, S.C. and Higuchi, W.I., Dissolution rates of high energy polyvinylpyrrolidone (PVP)-sulfathiazole coprecipitates, *J. Pharm. Sci.*, 58 (1969) 538–549.