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Rapid communication

An in vitro kinetic method for detection of precipitation of poorly soluble drugs

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Abstract

A simple in vitro method for the detection of precipitation using 96-well microplates and a SpectraMax Plus microtiter plate reader has been developed and described. The method requires only small amount of drug and is, therefore, potentially applicable in early pre-formulation. The method is based on opacity changes that occur with precipitation and yields several descriptive parameters, onset time (T_{onset}), maximum rate (V_{max}) and the time to reach V_{max} (T_{max}). Using these parameters, potential parenteral formulations can be ranked by their tendency to precipitate on dilution. We report use of this method and ranking of potential formulations of ricobendazole (RBZ), a poorly soluble anthelmintic, in various solvent systems. Detection at 500 nm was more sensitive than a wavelength of 550 nm and increased temperature (37 °C compared with 25 °C) accelerated precipitation. Results demonstrated the method was simple, descriptive and objective in the detection of precipitation of ricobendazole formulation on dilution and pH shift.

Keywords: Kinetic precipitation detection; Microtiter plate reader; Poorly soluble drugs; Turbidity; Onset time; Ricobendazole

Post-injection precipitation is an important concern for poorly soluble drugs formulated as injections at low or high pH or with co-solvent solubilisation. Drug precipitation tends to occur with pH neutralisation or co-solvent dilution by blood or extracellular fluid. Problems caused by drug precipitation at injection sites include pain on injection, inflammatory reactions and sometimes reduced drug bioavailability (Yalkowsky et al., 1998). Various in vitro methods have been developed to study precipitation of parenteral formulations, e.g. the dynamic injection model (Yalkowsky et al., 1983) and static serial dilution model (Li et al., 1998). Precipitation is determined by eye or by measuring opacity spectrophotometrically at wavelengths between 400 and 500 nm. In the dynamic method, the formulation is added into buffer or an infusion fluid through a specifically designed injector. Both flow rates can be adjusted to simulate the precipitation event

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that occurs on IV administration. The results using this model have been reported to correlate highly with phlebitis in test animals (Johnson et al., 2003). The static method is effective to measure the precipitation potential, amount and lag time due to retarded nucleation and crystal growth in super-saturated solutions (Alvarez-Nunez and Yalkowsky, 1999).

In this communication, we report a modified static method that allows measurement of precipitation kinetics at a range of dilution factors using a SpectraMax Plus microtiter plate reader. Only a small volume of the formulation is required, so that it may be useful in early pre-formulation studies and several parameters are generated to describe the precipitation process. We report application of this method in the development of a parenteral formulation of the anthelmintic ricobendazole (RBZ) which has an aqueous solubility 62 μg/ml (Wu et al., 2005).

RBZ hydrochloride (Transchem Ltd., India) solutions (5%, w/v) in various solvent systems were prepared (Table 1). An RBZ hydrochloride solution in water was used as a control (pH 1.5). All solvents were of analytical grade or suitable for use in parenteral products. Sorensen's phosphate buffer (SPB, pH 7.4, 0.1 M) filtered through 0.45 μm filters (Millipore S.A., France) was used as the diluent. Twelve 100 μl aliquots of each of the eight formulations were added to individual wells of a 96-well microtitre plate. Triplicate volumes (20, 30, 50 or 70 μl per well) of SPB were then added using an Eppendorf multi-channel pipetter (eight channel) giving progressive dilutions of the formulations. Dilutions across the 96-well plates took 30 s to complete. The dilution factor (DF) was defined as the ratio of the volume of formulation to the total volume (formulation + added SPB) (Li et al., 1998). Optical densities (OD; 500 and 550 nm) of wells were measured in the same order as the dilution process, using a SpectraMax Plus microtiter plate reader equipped with SoftMax Pro software (Molecular Devices). Then OD was measured every minute over 15 min with the samples being mixed by shaking for 3 s prior to each reading (reading points = 16). The experiments were conducted at 25 and 37 °C and the following parameters were obtained: (1) T_{onset} = onset time of detectable OD above initial reading, (2) V_{max}, the maximum slope of the kinetic display of OD versus time, expressed in milli-optical density units per minute (mOD/min) and (3) T_{max}, the time to reach V_{max}. Additionally, OD changes over time could be plotted to provide a graphical representation of the drug precipitation kinetics. For the optimisation of a parenteral formulation of RBZ, T_{onset} parameter was considered important as it represents the time at each dilution level that drug may be available in solution at concentrations above its aqueous solubility.

Data were obtained in a single 96-well microtiter plate for eight RBZ formulations (Table 1) diluted with SPB (DF 0.83, 0.77, 0.67 and 0.59). Table 1 shows representative kinetic parameters for formulations at a DF 0.59, which represents dilution of 100 μl formulation with 70 μl SPB ($n = 3$). As the dilution fraction increased, RBZ showed a lower tendency to precipitate with both T_{onset} and T_{max} increasing, and V_{max} decreasing, or becoming no longer detectable. Since the dilution process took 30 s to complete and the wells were read over 10 s in the same direction as dilutions were made, the "zero-time" error was less than 20 s.

Table 1
Precipitation of ricobendazole HCl aqueous solutions (RBZ 5%) after dilution with buffer (dilution factor 0.59 at 25 °C, the data are presented as mean ± S.D. ($n = 3$) at 500 nm)

Formula no.	Excipients	T_{onset} lag-time (s)	T_{max} (s)	V_{max}
F 1	[a]	151 ± 7	90 ± 0	47 ± 4
F 2	HP-β-CD (10%)	196 ± 4	120 ± 0	42 ± 3
F 3	HP-β-CD (15%)	514 ± 31	350 ± 62	17 ± 2
F 4	PG[b] (10%), *N*-methyl-2-pyrrolidone (10%)	189 ± 1	90 ± 0	27 ± 2
F 5	PG (10%), glycerol (10%)	78 ± 2	30 ± 0	33 ± 2
F 6	PG (10%), dimethyl sulphoxide (10%)	177 ± 14	110 ± 17	35 ± 5
F 7	PG (10%), polyethylene glycol 200 (10%)	163 ± 2	90 ± 0	41 ± 1
F 8	PG (10%), 2-pyrrolidone (10%)	180 ± 21	100 ± 17	38 ± 4

[a] Distilled water only.
[b] Propylene glycol.

At 25 °C, precipitation occurred in the control solution at all cases except at DF 0.83, whereas the co-solvent formulations (except for F 5) and 10% hydroxylpropyl-β-cyclodextrin (HP-β-CD, Roquette) containing formulation showed precipitation only at DF 0.67 and 0.59; 15% HP-β-CD formulation showed the lowest tendency to precipitate in this test with precipitation occurring only at DF 0.59. Prolonged T_{onset} and T_{max} and decreased V_{max} values were observed in these formulations compared to the control at each DF level (Table 1). In contrast F 5, containing glycerol, had a faster onset and rate of precipitation after dilution at all DF levels. These results demonstrated that the co-solvent (except for F 5) and cyclodextrin formulations retarded the onset and minimized the rate of RBZ precipitation on dilution, compared to the control formulation. HP-β-CD showed a dose-dependent effect on inhibition of RBZ precipitation.

A strong correlation was found between T_{onset} and T_{max} ($r = 0.991$, $P < 0.001$), the parameters which describe the lag time and amount of drug precipitation. Correlations between V_{max} and T_{onset} or T_{max} were not statistically significant ($P > 0.05$).

The effect of temperature on the precipitation was also investigated at a dilution factor of 0.77 and it was found that precipitation from formulations 2, 4, 6–8 occurred at 37 °C, but was not detectable at 25 °C. T_{onset} and T_{max} at 37 °C were significantly shorter ($P < 0.01$) and V_{max} were higher ($P < 0.05$) than that at 25 °C. This suggested that increase in the temperature increased the chance, or accelerated the process of drug precipitation from the solution. Although drug solubility increases with increasing temperature, the lower viscosity of formulation at higher temperature may play an important role in mixing or nucleation of the saturated solution in this test.

Various wavelengths (above 400 nm) have been used (Yalkowsky et al., 1983; Johnson et al., 2003). In 48 measurements (DF 0.67 and 0.59 at 25 °C), T_{onset} at 500 nm were 16.9 ± 3.9 s shorter than those obtained at 550 nm, but no significant difference in T_{max} or V_{max} ($P > 0.05$) were observed. This indicated the sensitivity of the detection of precipitation could be improved by using 500 nm provided no interfering absorbance occurs from formulation ingredients.

In conclusion, results demonstrated that the method is simple and rapid and produces several objective quantitative parameters for kinetic description of drug precipitation upon dilution or pH shift. It was found to be useful in formulation screening for the poorly water-soluble drug, ricobendazole. The method may be used to assess precipitation potential at slow mixing sites such as subcutaneous and intramuscular injections.

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