Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 698–704

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

A high-throughput spectrophotometric approach for evaluation of precipitation resistance

S. Chandran*, C. Gesenberg, J. Levons, M. Hubert, K. Raghavan

Bristol-Myers Squibb Co., 1 Squibb Drive, New Brunswick, NJ 08903, United States

ARTICLE INFO

Article history:
Received 18 April 2011
Received in revised form 6 July 2011
Accepted 11 July 2011
Available online 19 July 2011

Keywords:
Spectrophotometry
Turbidity
Toxicology formulation
High-throughput

ABSTRACT

Many drugs that come out of discovery today are extremely challenging to formulate because of their high hydrophobicity and low water solubility. A kinetic, spectrophotometric approach for the rapid evaluation of precipitation resistance of multiple solubilized formulations that can be run on a very small scale is proposed. Using this high-throughput approach, multiple formulations are screened for inhibition of precipitation upon dilution into an aqueous environment. Data generated using the *in vitro* spectrophotometric approach is comparable to the traditional chromatographic approaches. Similar approaches have been previously attempted, but in this study, the focus is on pharmaceutical development of solubilized formulations by evaluation of the events post precipitation as opposed to determining the moment at which precipitation is observed, which is usually the case in kinetic solubility measurements. The information garnered thus offers insights not just into amount of precipitation, but also to the size and nature of the precipitate. Overall, this technique offers the potential to change the approach to the development of toxicology vehicles and solubilized clinical formulations for oral administration.

1. Introduction

Over the past few decades, high throughput methodologies have permeated into the pharmaceutical discovery process with millions of small molecules being screened against various criteria such as solubility, potency, selectivity and ADME [1,2]. This intense selection pressure has led to a large number of modern drugs that are hydrophobic and very slightly soluble to practically insoluble in aqueous media. Consequently, this poses a considerable challenge to drug development in terms of ensuring satisfactory oral absorption. Given these very low solubilities, formulation efforts have revolved around ensuring oral absorption with the initial objective being to provide good exposure in preclinical studies in animal species. However, in today's development environment where rapid turn-around time is desired, it is often challenging to efficiently develop preclinical toxicology vehicles. In the past several years, high throughput experimentation techniques have also been developed by companies in order to assist formulation selection during drug development [2,3]. This is an emerging area and researchers are still attempting to develop high throughput platform technologies for formulation work. In the last two decades, novel high throughput technologies have been applied to the physico-chemical and formulation selection process [4]. High throughput UV methods for estimation of thermodynamic and kinetic solubility have been defined in literature [3,5–7]. There are UV based approaches for detecting precipitation of parenteral formulations after injection [8] and advanced screening assays for rapid identification of solubility-enhancing formulations with multiple case studies [9]. Of particular interest is a high-throughput approach used for discovery of a Cremophor EL-free paclitaxel formulation [10]. In addition, an *in vitro* kinetic method for detection of precipitation of poorly soluble drugs has been described. This can serve as a valuable technique for rank-ordering different formulations in terms of precipitation resistance [11].

In this article, the idea of using spectroscopic techniques for design of animal toxicology vehicles and advanced human clinical formulations is evaluated. Three model molecules, viz. 17β-estradiol, naproxen and camphor were chosen for this study. 17β-estradiol is almost insoluble in water, naproxen is practically insoluble in water and camphor is slightly soluble with a value of 1.25 mg/ml [12]. Due to the low solubilities of these molecules, formulators would face a challenge in providing satisfactory exposure were they to be orally administered. This problem is especially compounded during toxicology studies in early development where higher drug concentrations in formulation vehicle and wider dose range are expected, relative to clinically relevant formulations, in order to test for safety in animals. The use of a plate reader in formulation development has a role beyond what can be observed by the naked human eye. By numerically quantifying the degree of precipitation, it is possible to obtain some level of standardization. For example, there is the possibility of numerically quantifying precipitation as opposed to categorizing it into low versus moderate

* Corresponding author. Tel.: +1 732 227 5384; fax: +1 732 227 3782.
E-mail address: sachin.chandran@bms.com (S. Chandran).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.07.009

precipitation. This would lend researchers the ability to compare different formulations for precipitation resistance. In addition, such a technique would be easy to use and have the potential for rapid assaying compared to chromatography methods. The potential for significant cost, material and time savings is of extreme importance at this juncture in the pharmaceutical industry.

Finally, the method outlined in this article uses turbidity as a measure which is not a property of the individual molecules being tested. This implies that this approach can be suitably extended to other drug candidates with relative ease.

2. Materials and methods

17β-estradiol, naproxen and camphor were obtained from Sigma–Aldrich (St. Louis, MO). Polyethylene glycol (PEG) 400, polyvinylpyrrolidone (PVP) K-12 and K-30, Pluronic F127 (F127) and d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS) were sourced internally from Bristol-Myers Squibb (BMS) (New Brunswick, NJ). The spectrophotometer used was a Biotek (Winooski, VT) Powerwave XS2 with Gen 5.0 software. The Ultra Performance Liquid Chromatography (UPLC) system used for analysis was a Waters (Milford, MA) Acuity with a tunable detector and a 50 mm AcQuity UPLC BEH C18, 1.7 μm column. A Lasentec Focused Beam Reflectance Measurement (FBRM) S400 probe by Mettler Toledo (Columbus, OH) was used for particle size determination.

2.1. Preparation of drug solutions

The following stock solutions were initially prepared; 20% F127 (w/w) in PEG 400, 10% (w/w) PVP-K12 in PEG 400, 10% (w/w) PVP-K30 in PEG 400 and 0%, 1%, 2%, 5%, and 20% (w/w) TPGS in PEG 400. Slight warming and agitation was required to dissolve some of the excipients in PEG 400.

Three separate 3 mg/g solutions of 17β-estradiol were prepared in neat PEG 400, 20% F127 (w/w) in PEG 400 and 20% (w/w) TPGS in PEG 400, respectively. Similarly three separate 20 mg/g solutions of naproxen were prepared in PEG 400, 10% (w/w) PVP-K12 in PEG 400 and 10% (w/w) PVP-K30 in PEG 400. Finally, four separate 20 mg/g solutions of camphor were prepared in stock solutions of 0%, 1%, 2% and 5% (w/w) TPGS in PEG 400. Gentle heating and agitation was required to dissolve the test molecules in all formulations. After preparation, the solutions were stored at 40 °C and used within an hour.

2.2. Precipitation resistance studies using the plate reader

In separate experiments utilizing each test molecule individually, 100 μl of the drug solution to be tested was transferred into a well of a 96-well polypropylene plate. Each individual stock solution was pipetted into three separate wells, so as to run each sample in triplicate. The experiment was initiated by addition of an equal volume of deionized water to each well using a multichannel pipette. The 96-well plate was then placed on the spectrophotometer and samples read at 500 nm [13,14]. At 500 nm, none of the molecules screened had an intrinsic absorbance as checked by the plate reader, thereby implying that turbidity was the only contributor to the observed signal. The incubation temperature within the samples chamber was maintained at 37 °C for the duration of the experiment. The samples were also shaken between each reading. All analyses and calculations were carried out within Gen 5.0.

2.3. Lasentec analysis of particle size

The Lasentec probe was equilibrated in 20 g of 20 mg/g solution of camphor in PEG 400 in a 50 ml flask under agitation at 400 rpm at room temperature and data collection was initiated. At the start of the experiment, 20 ml of deionized water was added to the flask. Particle size distribution data using Lasentec Focused Beam Reflectance Measurement (FBRM) was collected every 15 s for approximately 30 min. The experiment was repeated in identical fashion for a 20 mg/g solution of camphor in 1% (w/w) TPGS in PEG 400. The determined particle size would be represented by chord length.

2.4. Determination of precipitation resistance potential of camphor formulations by chromatography

To 50 mg of 20 mg/g solution of camphor in 0%, 1%, 2% and 5% (w/w) TPGS in PEG 400 was added 50 ml of deionized water to replicate the similar dilution of 1:1 that was used for the plate reader experiment. At 15 min and 60 min, approximately 3 ml of sample was withdrawn and filtered through a 0.45 μm polyvinylidene fluoride (PVDF) membrane filter. The initial 2.5 ml of filtrate was discarded and then 100 μl of filtrate was collected and diluted 10-fold into 50% acetonitrile in water (v/v) and analyzed by UPLC. Camphor standard curves were prepared in 50% acetonitrile in water (v/v).

3. Results and discussion

3.1. Comparing different excipients in formulations for 17β-estradiol and naproxen

The UV plate reader has traditionally been used for spectrophotometric determination of absorption values. Theoretically, the absorption value at a particular wavelength is defined as the logarithm of the inverse of transmittance. However, in these studies, an alternative use of the UV plate reader in determination of the precipitation resistance of pharmaceutically relevant formulations is outlined. It should once again be noted that the wavelength of 500 nm used in these studies is well above the range of absorption of all the molecules being studied thus preventing any interference. The observed values of absorbance are solely due to the occlusion of light as it passes through the well containing formulations that have precipitated. To satisfactorily evaluate the precipitation data from the plate reader, it is imperative to understand the contribution of occlusion to the process. By observations made with the naked eye, it is known that formulations of 17β-estradiol and naproxen in PEG 400 will immediately show precipitation upon dilution in water. It is also known that over time, these systems will become relatively clear. However, nothing is known about the kinetics or mechanism of this process as it would be difficult to follow it with the naked eye.

The plate reader contributes to this effort by continuous measurement of absorbance. The observed absorbance is due to the occlusion of the passage of light upon precipitation which in turn decreases transmittance, thereby increasing absorbance. This is outlined in Figs. 1 and 2 for 17β-estradiol and naproxen, respectively. In all cases, the formulations of these test molecules in neat PEG 400 (Figs. 1A and 2A) show high initial absorbance followed by gradual lowering with time. The profiles of the absorption curves change slightly in the presence of other excipients. All readings are in triplicate with similar absorbance profiles for each well. In the case of 17β-estradiol, the presence of 20% F127 (w/w) in the PEG 400 maintains turbidity over a period of almost 3 h (Fig. 1B). With 20% (w/w) TPGS in the formulation, almost no turbidity is detected for the entire length of time monitored (Fig. 1C). Similarly, for naproxen, a stark difference is observed between the precipitation profiles with PVP-K12 and PVP-K30 in the formulation. PVP is generally known in literature as a good crystallization inhibitor

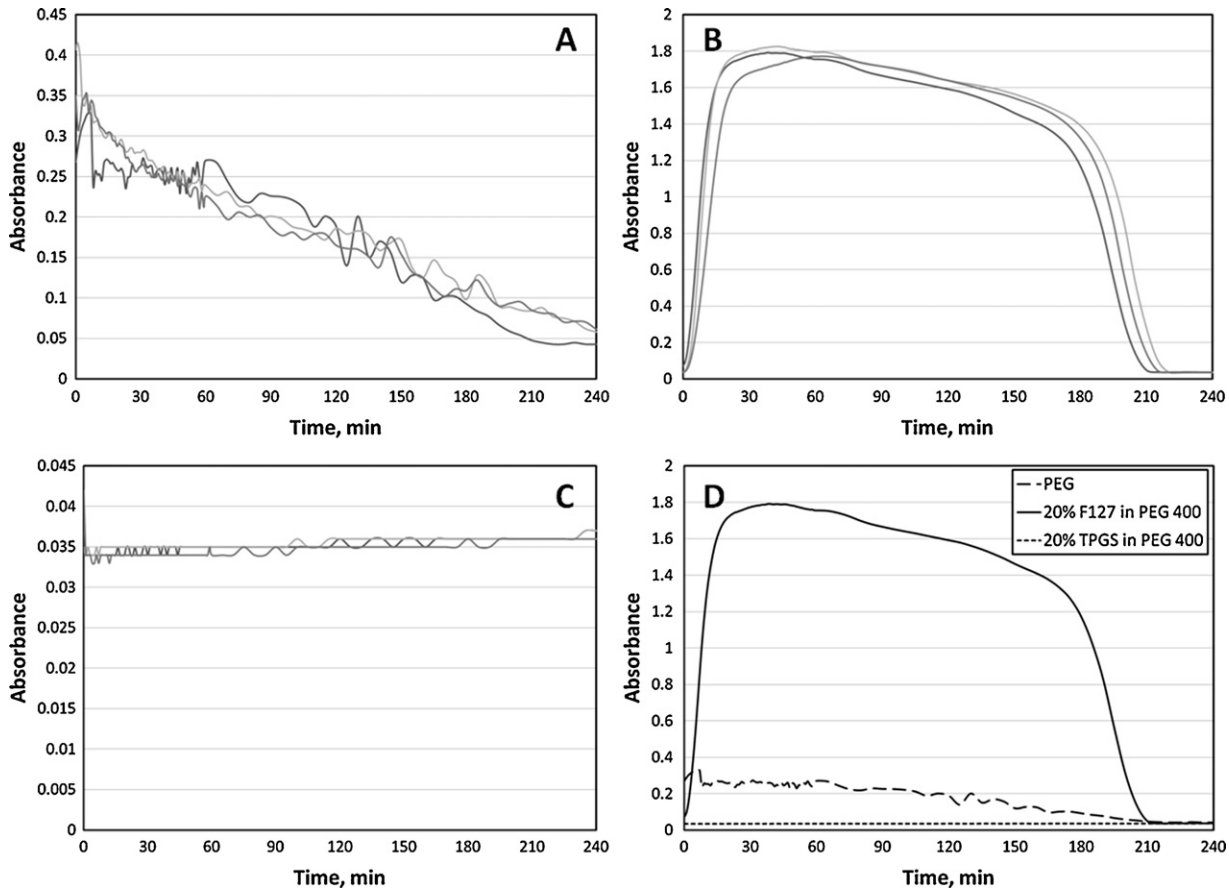

Fig. 1. Precipitation data for 17β-estradiol in (A) PEG 400, (B) 20% F127 (w/w) in PEG 400, and (C) 20% TPGS (w/w) in PEG 400. (D) Compares a representative sample for each case.

[15], and this technique seems to be able to differentiate the effect between two PVP chain lengths. In the case of both PVP-K12 (Fig. 2B) and PVP-K30 (Fig. 2C), the maximum absorbance is at around 8 h, but the time taken for the absorbance to fall to close to zero is 12–14 h for PVP-K12 versus 20–24 h for PVP-K30. Figs. 1D and 2D respectively compare the three different formulations for 17β-estradiol and naproxen that were evaluated. In the case of both test molecules, there is a considerable difference in the precipitation profiles with the use of different excipients. For 17β-estradiol, the TPGS-based formulation provides the least absorbance values over the time period whereas for naproxen, the PVP-K30-based formulation maintains turbidity in the well for the longest possible time.

The authors hypothesize that the initial precipitation of fine particles causes a strong blockage of light and consequent rapid increase in absorbance. Subsequent agglomeration of particles then leads to increasing particle size and settling; this would result in a greater amount of light being able to pass through the cross section of the well, thus increasing transmittance, consequently decreasing absorbance. This would imply that in the case of 17β-estradiol and naproxen, the formulations containing TPGS and PVP-K30, respectively would be the best at providing precipitation resistance.

3.2. Comparing the same excipient, TPGS in camphor formulations

To determine the effects of different amounts of the same excipient, camphor was screened using varying amounts of TPGS in the formulation. Fig. 3 shows the precipitation data for camphor. It can be clearly observed that in the presence of 2% and 5% (w/w) TPGS in PEG 400, the increase in absorbance in the first 30 min is lower, as compared to the 1% and 0% (w/w) TPGS in PEG 400 system. It can therefore be concluded that the precipitation of camphor at a 1:1 dilution with water is considerably lower in the samples with 2% and 5% (w/w) TPGS in PEG 400. This indicates the precipitation inhibiting effect of TPGS. Interestingly, the neat PEG 400 system demonstrates a sharp initial increase followed by a rapid loss of absorbance. This data is similar in nature to what was observed for 17β-estradiol and naproxen. The high initial absorbance for the PEG formulation followed by the lowering of absorbance could be suggestive of initial precipitation formation followed by agglomeration, and possibly crystallization. This effect decreases with increasing amount of TPGS which suggests that adding TPGS is beneficial in providing crash resistance.

3.3. Agglomeration as observed by Lasentec FBRM

To validate the above claim that agglomeration leads to lowering of the absorbance signal, an orthogonal technique unrelated to the UV plate reader was used. FBRM is a probe-based technique that can be used for real-time measurement of particle and droplet size in concentrated suspensions and emulsions without the need for sample extraction and preparation [16]. Since the systems being considered in this study are fairly concentrated, FBRM is an ideal technique given the requirements. The two formulations compared were 20 mg/g camphor in PEG 400 with and without 1% TPGS (w/w). The hypothesis is that the increasing absorbance is due to precipitation of the drug and the subsequently decreasing absorbance is due to agglomeration of larger particles. In the case of the 1% (w/w) TPGS system, the particle size distribution shows most particles to

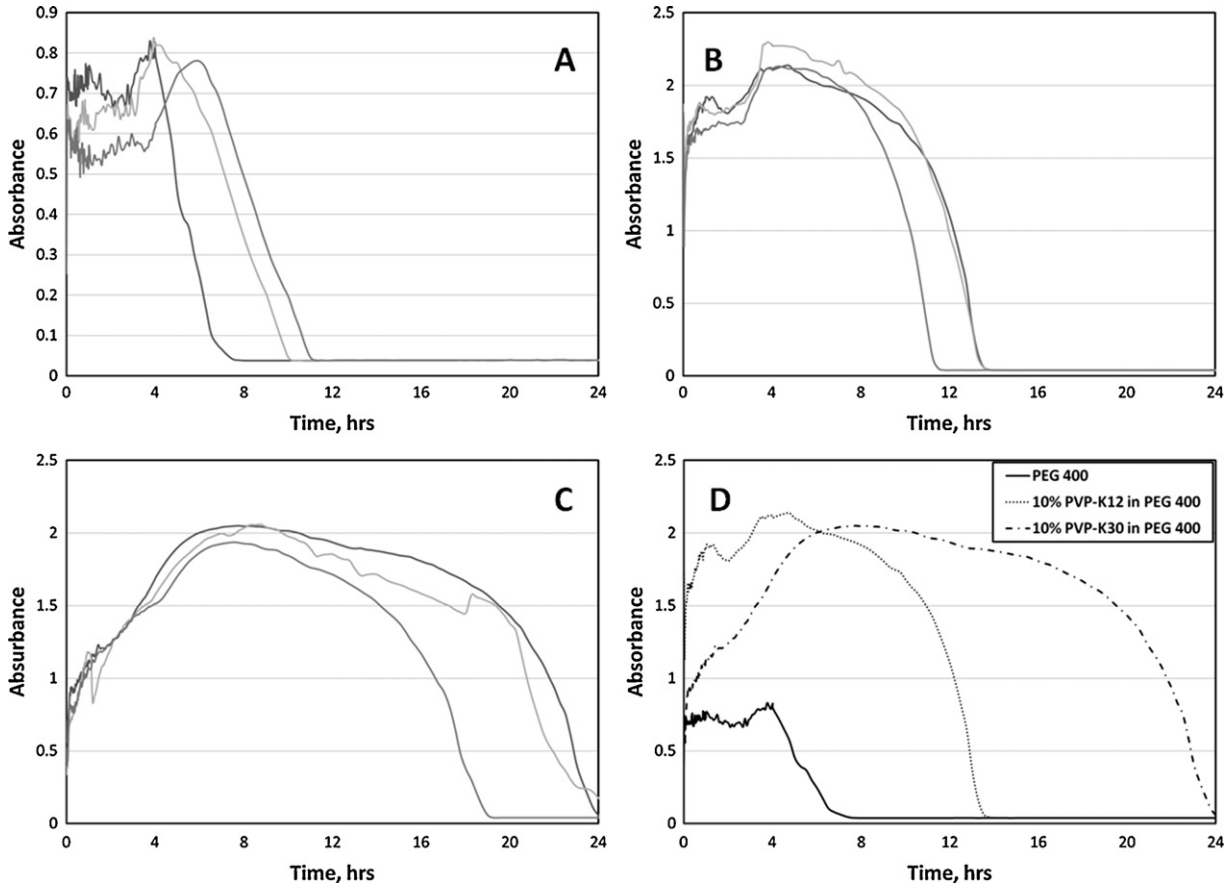

Fig. 2. Precipitation data for naproxen in (A) PEG 400, (B) 10% PVP-K12 (w/w) in PEG 400, and (C) 10% PVP-K30 (w/w) in PEG 400. (D) Compares a representative sample for each case.

be in the 20–30 μm range (Fig. 4A) whereas the neat PEG 400 system shows particles mostly in the 100–200 μm (Fig. 4B). This effect is certainly more pronounced at 20 min where the difference in the particle size can be observed between the two formulations (Fig. 4C). If the particle counts were to be examined, at 10 min, most particles in the formulation with TPGS are below 100 μm in size whereas most particles in the PEG 400 formulation are between 100 and 1000 μm in size (Fig. 4D). It has to be noted that no bubbles were observed in the formulations as the agitation was not very rapid.

FBRM data therefore suggests that the particle sizes of the precipitate from the PEG formulation are much higher than those from the TPGS formulation. If the system precipitates and crystallizes rapidly, a greater cross-section of the well is open to the transmission of light, and therefore, the absorbance values are low. However, if the precipitate is of a fine nature, or the system forms a milky suspension upon dilution, the turbidity is high, which leads to low transmission and higher values of absorbance. It is this difference in particle size that leads to different absorbance curves on the UV plate reader. The TPGS formulation is able to maintain the precipitate at a lower particle size compared to the PEG 400 which leads to a higher turbidity and consequently, a higher absorbance for a longer period of time. This is consistent with the hypothesis that has been presented.

3.4. Comparison with reverse-phase chromatography data

The more commonly used method to measure the degree of precipitation of drug substance from solution formulations after dilution into an aqueous environment is to filter the samples after certain periods of time and analyze the filtrate by chromatography. To evaluate whether the qualitative data obtained from the plate reader resulted in data that would be comparable to the more time and resource-consuming UPLC approach, a comparison between the different TPGS formulations was carried out by a chromatography analysis (Fig. 5). The degree of supersaturation is based on a solubility value of 6 mg/ml for camphor in 50% (w/w) PEG 400 (experimentally determined). Based on the chromatography data, at 15 min the camphor had completely precipitated out in

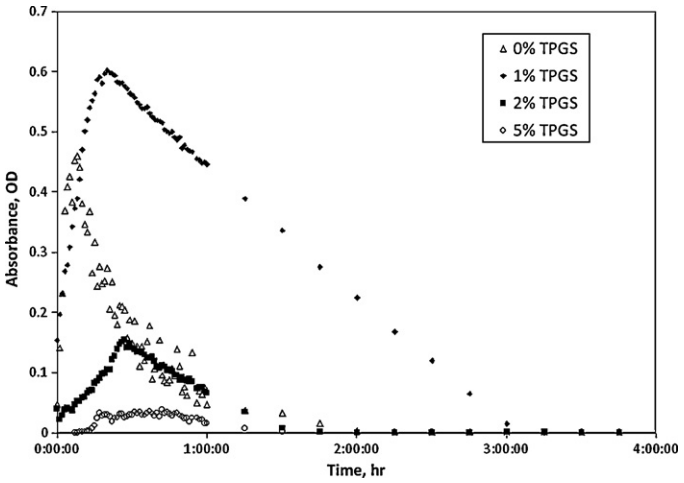

Fig. 3. Kinetic absorption spectra of camphor formulations in 0%, 1%, 2% and 5% (w/w) TPGS in PEG 400 diluted in water.

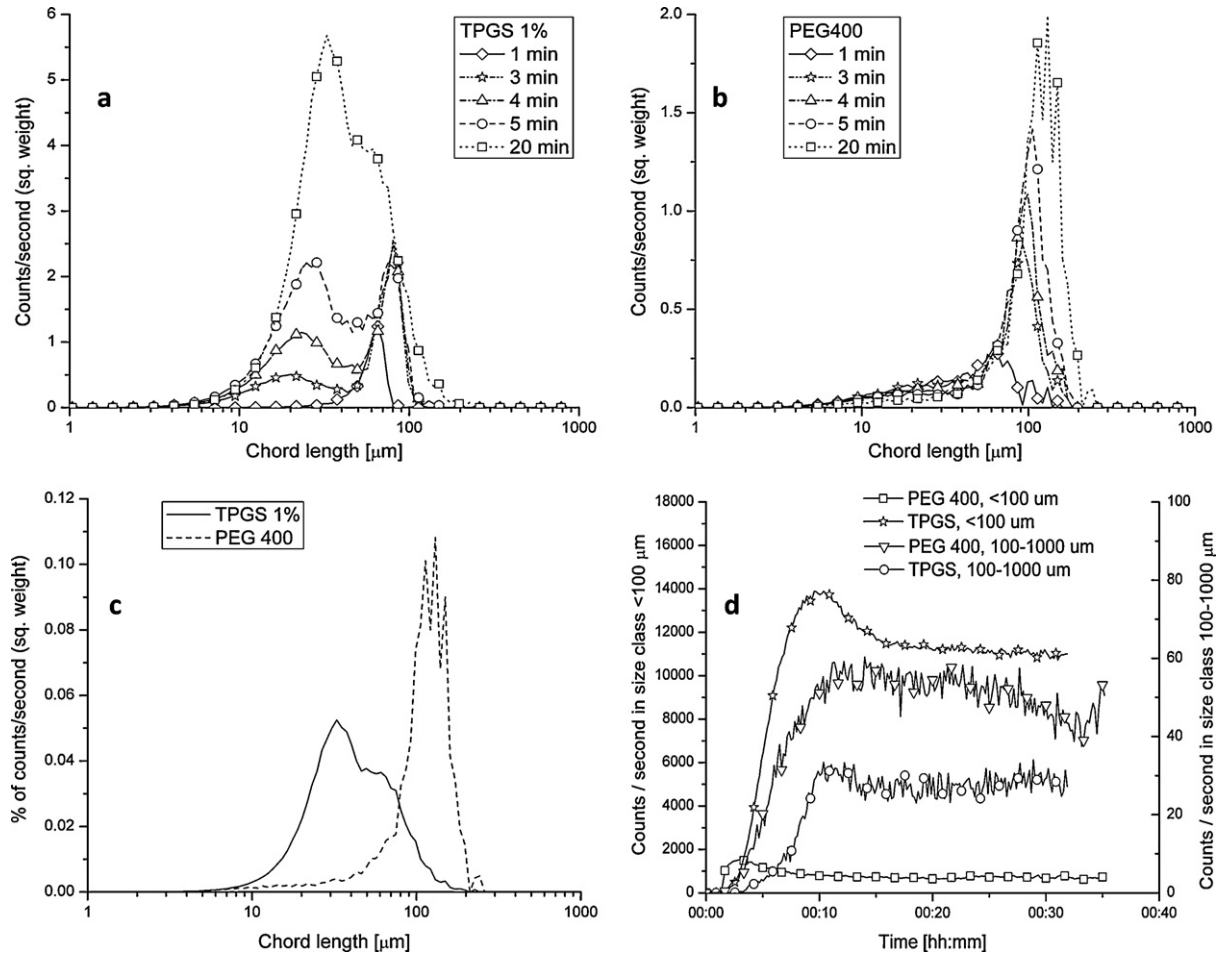

Fig. 4. Lasentec FBRM analysis to evaluate particle size upon dilution of camphor formulations in water. (A) Change in particle size with time for camphor in 1% TPGS in PEG 400 system. (B) Change in particle size with time for camphor in PEG 400 system. (C) Comparison of particle size distribution at 20 min between camphor in neat PEG 400 and camphor in 1% TPGS in PEG 400. (D) Trend lines for particle sizes.

the system without the TPGS. A beneficial precipitation inhibiting effect was observed with increasing amounts of TPGS. At the 5% (w/w) TPGS level, the system was observed to have very high precipitation resistance potential with almost 50% super-saturation remaining after 1 h. Based on the UV plate reader, the 5% (w/w) TPGS in PEG 400 was identified as the best formulation for crash resistance. This qualitative data is reflected in the data obtained from the traditional UPLC method.

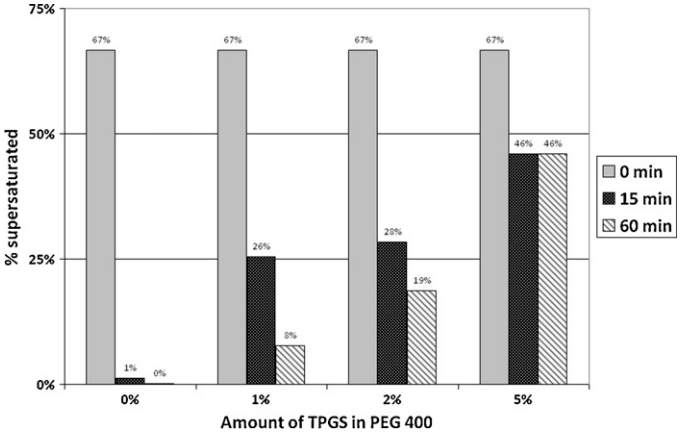

Fig. 5. UPLC analysis demonstrating increasingly supersaturated camphor with respect to increasing amount of TPGS in vehicle.

3.5. Comparison of UV plate reader data with FBRM and chromatography

Using the three separate molecules that were screened in this study, it is evident that the spectrophotometric approach can tease apart differences in precipitation behavior upon dilution between formulations based on the simple assessment of turbidity. Though this concept has been examined earlier [11], an improved understanding of the precipitation process and the corresponding interpretation of the data is proposed in this manuscript. An important aspect is an enhancement in the quality of the data by considering the kinetic process. If the data for 17β-estradiol (Fig. 1D) were evaluated at only one time point (i.e. 4 h) as typically done when testing for degree of precipitation by chromatography, all formulations would look identical, and no significant difference would be detected between the formulations containing F127 or TPGS in PEG 400. However, in reality, large differences in the precipitation behavior are observed between the three formulations as observed by the plate reader.

The plate reader allows for rapid analysis at multiple time points without additional sample preparation or consumption. By looking at the kinetic data at small intervals over a long period, it is possible to arrive at more physiologically meaningful conclusions. For example, in the case of naproxen, the PVP-K30 would certainly be a better choice of excipient as compared to PVP-K12 since it provides a longer time period where the precipitate exists in the form of small particles without crystallizing into larger ones (Fig. 2D).

In the case of camphor, in conjunction with Lasentec FBRM work, it can be easily concluded that in the absence of TPGS, camphor is not only precipitating in quick fashion, but also agglomerating rapidly. This is clearly reflected in the spectrophotometric output where the suspension resulting from the PEG solution never reaches the high absorbance values that are obtained in case of the 1% (w/w) TPGS formulation. As mentioned previously, the rapid attenuation of the absorbance signal in the case of the neat PEG 400 formulation is due to agglomeration that opens up more pathways for light to pass through, compared to a more evenly dispersed suspension of fine particles that blocks out more light resulting from a solution containing 1% TPGS.

The dilution used for the plate reader experiments is not of direct physiological relevance. Despite that, the rank ordering of the quality of the formulations is maintained when compared with the UPLC approach. The plate reader approach is quick, whereas the traditional chromatography method is much more laborious, and time and resource-consuming.

4. Conclusions

In today's environment, the doses for drug molecules especially for evaluation of preclinical safety are being pushed so that toxicology groups and clinicians can get the best possible read for determination of a safe, yet useful dose range for clinical trials in humans. At this time, the onus is on development teams to provide the best possible precipitation resistant formulations. The humble UV plate reader has been traditionally used for concentration determination or evaluation of reaction rates by colorimetric assays. In this article, the authors put forth another use, viz. the rapid evaluation of pharmaceutically relevant solubilized formulations by measuring turbidity using a UV plate reader.

There exists considerable literature where kinetic solubility is calculated by a turbidimetric approach [7,17–20]. However, there are distinct differences in what is presented here versus what is reported in the literature. This is of great importance to pharmaceutical scientists. This manuscript provides important considerations in the development of toxicology formulations, especially for a wide range of doses, in that the drug maintain its supersaturation and the duration to which this supersaturation is maintained, i.e. the 'hang-time' of the formulation. These have a direct bearing on the extent of absorption of the drug. While the literature provides techniques to measure the point at which precipitation occurs, what is novel about this method is that it gives a measure of the time course of precipitation that can be used to analyze and interpret relevance to an in vivo situation in order to assess performance. Commonly used vehicles that are considered acceptable for preclinical toxicology testing from a safety point of view are utilized (i.e. not vehicles like methanol, DMSO, etc.). This will provide a great guide to pharmaceutical scientists, especially those in industry, on generating meaningful data utilizing vehicles of practical utility. Examination of the nature of the precipitate utilizing meaningful vehicles, and the associated particle size distribution is of great importance to get a read on bio-performance. If the precipitate is of extremely small particle size, there will always be a possibility of re-dissolving in the GI milieu and eventually get absorbed, which is especially true for drugs that exhibit dissolution rate limited absorption.

The fundamental novelty of this technique is interpreting the time versus absorbance plot into various stages of precipitation. In the references in literature that examine kinetic solubility, a discrete point to determine the instance of precipitation is identified [7,17,19]. This work goes beyond this concept to determine if precipitation occurs within the time scale of the experiment and the point when substantial precipitation occurs. In addition, a relative assessment of the magnitude of the differing amounts of precipitation between the formulations and the point of agglomeration is also provided. This setup merely recreates on a smaller scale, the processes that are happening when concentrated formulations hit the GI tract.

Furthermore, the evaluation by UPLC and FRBM is to use orthogonal techniques to qualify the data obtained from the plate reader. Liquid chromatography is the current gold standard for analysis of potency in pharmaceutical development and comparison of the plate reader data with UPLC adds greater credence to the claims that it is possible to monitor precipitation resistance and therefore design better formulations by the use of the plate reader.

Though the approach is relatively simple, it fulfils an unmet need for rapid evaluation of multiple formulations and indicates that UV spectrophotometry is a viable technique for evaluation of precipitation resistance on a very small scale. Samples for 17β-estradiol and naproxen that were run in triplicate provided similar data speaking to the robustness and lack of variability associated with the technique. Given the ease of adapting the 96-well plate to automation and high-throughput processes, it is hypothesized that it is possible to develop animal toxicology vehicles by screening a wide range of vehicles and precipitation inhibitors with rapid turn-around times by using this simple screen for determination of the efficiency of various formulations for precipitation resistance. The data from this screen would be qualitative enough to provide a rank-ordering of the best formulations that could further be evaluated by more traditional approaches such as chromatography or dissolution. While the data summarized in this article represents an initial validation of the feasibility of the technology, experimental work with more drug like compounds and dilution ratios that represent an in vivo situation more closely are ongoing and will be subject for future publications.

Acknowledgements

The authors wish to acknowledge B. Vig, M. Hussain and A. Thakur for critical input and suggestions.

References

[1] G.W. Caldwell, Z. Yan, W. Tang, M. Dasgupta, B. Hasting, ADME optimization and toxicity assessment in early- and late-phase drug discovery, Curr. Top. Med. Chem. 9 (2009) 965–980.
[2] D.M. Goldstein, N.S. Gray, P.P. Zarrinkar, High-throughput kinase profiling as a platform for drug discovery, Nat. Rev. 7 (2008) 391–397.
[3] E.H. Kerns, L. Di, G.T. Carter, In vitro solubility assays in drug discovery, Curr. Drug Metab. 9 (2008) 879–885.
[4] C.R. Gardner, O. Almarsson, H. Chen, S. Morissette, M. Peterson, Z. Zhang, S. Wang, A. Lemmo, J. Gonzalez-Zugasti, J. Monagle, J. Marchionna, S. Ellis, C. McNulty, A. Johnson, D. Levinson, M. Cima, Application of high throughput technologies to drug substance and drug product development, Comput. Chem. Eng. 28 (2004) 943–953.
[5] J. Alsenz, M. Kansy, High throughput solubility measurement in drug discovery and development, Adv. Drug Deliv. Rev. 59 (2007) 546–567.
[6] B. Bard, S. Martel, P.A. Carrupt, High throughput UV method for the estimation of thermodynamic solubility and the determination of the solubility in biorelevant media, Eur. J. Pharm. Sci. 33 (2008) 230–240.
[7] T. Heikkilä, M. Karjalainen, K. Ojala, K. Partola, F. Lammert, P. Augustijns, A. Urtti, M. Yliperttula, L. Peltonen, J. Hirvonen, Equilibrium drug solubility measurements in 96-well plates reveal similar drug solubilities in phosphate buffer pH 6.8 and human intestinal fluid, Int. J. Pharm. 405 (2011) 132–136.
[8] S.H. Yalkowsky, S.C. Valvani, B.W. Johnson, In vitro method for detecting precipitation of parenteral formulations after injection, J. Pharm. Sci. 72 (1983) 1014–1017.
[9] W.G. Dai, C. Pollock-Dove, L.C. Dong, S. Li, Advanced screening assays to rapidly identify solubility-enhancing formulations: high-throughput, miniaturization and automation, Adv. Drug Deliv. Rev. 60 (2008) 657–672.
[10] H. Chen, Z. Zhang, C. McNulty, C. Olbert, H.J. Yoon, J.W. Lee, S.C. Kim, M.H. Seo, H.S. Oh, A.V. Lemmo, S.J. Ellis, K. Heimlich, A high-throughput combinatorial approach for the discovery of a Cremophor EL-free paclitaxel formulation, Pharm. Res. 20 (2003) 1302–1308.
[11] Z. Wu, I.G. Tucker, M. Razzak, N.J. Medlicott, An in vitro kinetic method for detection of precipitation of poorly soluble drugs, Int. J. Pharm. 304 (2005) 1–3.

[12] J. O'Neil, A. Smith, P.E. Heckelman, The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals/Maryadele, Merck, Rahway, NJ, USA, 2001.
[13] K.N. Pearce, J.E. Kinsella, Emulsifying properties of proteins: evaluation of a turbidimetric technique, J. Agric. Food Chem. 26 (1978) 716–723.
[14] F.-S.D. Xiao-Nan Huang, B. Zhang, J.-Y. Zhao, Z.-C. Li, Acid-labile, thermoresponsive (Meth)acrylamide polymers with pendant cyclic acetal moieties, J. Polym. Sci. A: Polym. Chem. 46 (2008) 4332–4343.
[15] K. Khougaz, S.D. Clas, Crystallization inhibition in solid dispersions of MK-0591 and poly(vinylpyrrolidone) polymers, J. Pharm. Sci. 89 (2000) 1325–1334.
[16] T. Allen, Particle Size Measurement, in: 4th ed., Chapman and Hall, New York, 1990.
[17] C.D. Bevan, R.S. Lloyd, A high-throughput screening method for the determination of aqueous drug solubility using laser nephelometry in microtiter plates, Anal. Chem. 72 (2000) 1781–1787.
[18] K.A. Dehring, H.L. Workman, K.D. Miller, A. Mandagere, S.K. Poole, Automated robotic liquid handling/laser-based nephelometry system for high throughput measurement of kinetic aqueous solubility, J. Pharm. Biomed. Anal. 36 (2004) 447–456.
[19] Y.C. Lee, P.D. Zocharski, B. Samas, An intravenous formulation decision tree for discovery compound formulation development, Int. J. Pharm. 253 (2003) 111–119.
[20] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 23 (1997) 3–25.