

Stabilizing Kavalactone Dispersions Utilizing Common Emulsifiers

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Piper methysticum, commonly known as kava, is a medicinal herb that is commonly used in traditional ceremonies and medical practices throughout the Pacific. The main bioactive compounds within kava are the kavalactones which can be used for the treatment of insomnia and anxiety; however, they have low bioavailability due to their poor water solubility. In order to increase the water solubility of kavalactones, the common emulsifiers, polysorbate 20 and polysorbate 80, were tested at different concentrations to determine the ideal kava dispersion system. The dispersion systems (containing 5.8% w/w kava powder) were mixed using a high-shear mixer prior to centrifugation to remove the sediment. The stability for each sample was then determined by measuring the particle size, zeta potential, and polydispersity index (PDI) utilizing a Malvern Zetasizer Nano ZS. As indicated by the results, kava dispersions with a 1.5% (w/w) concentration of polysorbate 80 managed to reduce particle size the most with an average particle diameter of 41.5 nm, which is 196.7 nm smaller than the average control particle size. However, the PDI for this sample was too high to be considered stable. The sample that exhibited the most stability was the 0.5% (w/w) concentration of polysorbate 20. This sample managed to maintain a particle size of 130.4 nm and a PDI of 0.44 over time. These results are a positive indication that nonionic emulsifiers, including polysorbate 20, at lower concentrations are suitable emulsifiers for kavalactone stabilization.

Keywords: kava, aw, kavalactones, emulsion, bioavailability, benzodiazepines, polysorbate 20, polysorbate 80, anxiety, insomnia

Introduction

Anxiety is one of the most common mental illnesses in the world. This ailment causes a variety of symptoms including fatigue, stomachaches, restlessness, headaches, mental

confusion, muscle aches, irritability, and insomnia (Dorner & Mittendorfer-Rutz, 2017). To treat anxiety disorders, patients are often administered benzodiazepines, commonly known as “benzos,” which are formed from the fusion of a benzene ring and a diazepine ring. Common commercial benzodiazepines



I am Stryder Williams, a student majoring in Biology (BS) and minoring in Psychology. This research project was completed under the mentorship of Dr. Kacie Ho as part of the IDeA Networks of Biomedical Research Excellence (INBRE) Student Research Experience. With this publication, I aspire to expand the literature that is currently available on improving the efficacy of kava for the treatment of anxiety and insomnia. In doing so, I hope to encourage more research into naturalistic alternatives to pharmaceuticals for the treatment of common mental disorders.

include Xanax, Ativan, Valium, and Restoril (Shinomiya et al., 2005). These medications work by enhancing the activity of the γ -Aminobutyric acid (GABA) neurotransmitter. This chemical in the brain reduces neuronal excitability throughout the nervous system, creating a sense of calmness, relaxation, and drowsiness. However, by enhancing GABA, benzodiazepines also have a variety of unintended side effects such as light-headedness, dizziness, muscle weakness, memory problems, and slurred speech. In addition, high-potency benzodiazepines, such as clonazepam, have the potential to cause dependence among their users (Möhler et al., 2002). If overused, benzodiazepines can commonly cause overdoses, which result in over 12,000 deaths each year (National Institute on Drug Abuse, 2023). Because of this, there has been an increasing interest in finding alternatives to benzodiazepines for treating both anxiety and insomnia. One of these alternatives is the medicinal plant kava (Pittler and Ernst, 2000). Similar to benzodiazepines, kava enhances GABA neurotransmitters resulting in feelings of relaxation. In addition, kava also inhibits noradrenaline uptake, which is involved in the regulation of mood and provides relief from depression and chronic pain (Bilia et al., 2002). However, unlike benzodiazepines, kava is nonaddictive and tends to have little to no side effects in individuals who consume them (Lasme et al., 2008).

Within kava, the main bioactive compounds that contribute to the plant's psychoactive effects are the kavalactones (Pittler and Ernst, 2000). These molecules are a class of cyclic esters (lactones) that are unique to the roots of kava plants (Siméoni and Lebot, 2002). So far, eighteen kavalactones have been identified and six (kavain, methysticin, dihydrokavain, dihydromethysticin, yangonin, and desmethoxyyangonin), are considered the main kavalactones due to their increased abundance and pharmacological importance (Zou et al., 2004). These compounds are known to provide numerous beneficial effects including anti-convulsion, relaxation, euphoria, pain relief, neuroprotection, analgesia, and reduction of menopausal symptoms (Wang et al., 2015). In prior studies, sleep-deprived rats that were administered 300 mg of kavalactone extracts for a week witnessed a notable decrease in sleep latency. These effects were comparable to current pharmaceutical drugs which are used for the treatment of insomnia (Shinomiya et al., 2005). In addition, individuals that were administered 120 mg of kava daily for six weeks reported a notable reduction in stress on a visual analog scale that measured stress levels in terms of social, personal, and life events (Wheatley, 2001). Even though kava demonstrates the ability to be used as an anxiolytic drug, this herb is still not as effective when compared to commonly used benzodiazepines (Sarris et al., 2012). This may be due to the fact that the kavalactones are quite hydrophobic and as a result are poorly water-soluble. Because of this, kavalactones have poor bioavailability and very few actually enter the bloodstream. Consequently, the vast majority of kavalactones are

excreted from the body before they can provide any beneficial effects (Pescitelli et al., 2010).

In order to increase the water solubility of kavalactones and thus their bioavailability, the common emulsifiers polysorbate 20 and polysorbate 80 were tested for their ability to stabilize kava dispersion systems consisting of kava root powder and water. Polysorbate 20 and 80 are nonionic surfactants derived from the ethoxylation of sorbitan fatty acid esters. Both of these surfactants are from the polysorbate family and have similar chemical structures. However, polysorbate 80 is slightly bulkier than polysorbate 20, thus causing differences in their chemical properties (Chou et al., 2005). Despite these slight differences, both of these synthetically derived emulsifiers are biocompatible, relatively cheap to produce, and exhibit high surface activity (Moore, 1984). Because of this, they are often used in foods and drugs for long-term shelf stabilization (Chou et al., 2005). In addition, emulsifiers from the polysorbate family have the ability to improve the solubility of poorly soluble drugs thus enhancing their penetrability and bioavailability (Nielsen, 2016). Prior studies utilizing nonionic emulsifiers to increase the solubility of hydrophobic drugs have seen a 200% increase in relative bioavailability following the encapsulation of the pharmaceutical within an emulsion system (Dollo et al., 2003). Considering this, polysorbate 20 and 80 have the potential to stabilize the hydrophobic and hydrophilic interface within a kava dispersion system, therefore, making the kava more soluble (Chou et al., 2005). In doing so, this has the potential to improve the quantity of kavalactones that have an effect on the body, thus increasing kava's efficacy for the treatment of anxiety and insomnia (Pittler and Ernst, 2000).

To determine emulsion suitability, first, the physical stability of the emulsion system has to be determined. Physical stability refers to the ability of an emulsion to maintain its structure over time without any phase separation. This is a prerequisite to long-term shelf stability and chemical stability within the gastrointestinal tract. If the emulsion is not physically stable, it may "break" or undergo phase separation in which the hydrophobic particles will become insoluble again (Yao et al., 2013).

To determine physical stability, the particle size, polydispersity index (PDI), and zeta potential need to be analyzed for each sample. The particle size measures the average nanoparticle diameter within the dispersal system. In general, smaller particles are desired as their smaller sizes decrease the likelihood of interactions and aggregation within the medium which would break the emulsion (Chen et al., 2022). Comparatively, PDI is a measurement of the heterogeneity of particle size within the system. This measurement ranges from 0.0 (for a completely uniform sample with no variance in particle size) to 1.0 (for a system with no uniformity in which all the particles are of different sizes). Ideally, the colloidal system should be monodisperse with a low PDI in which the particles

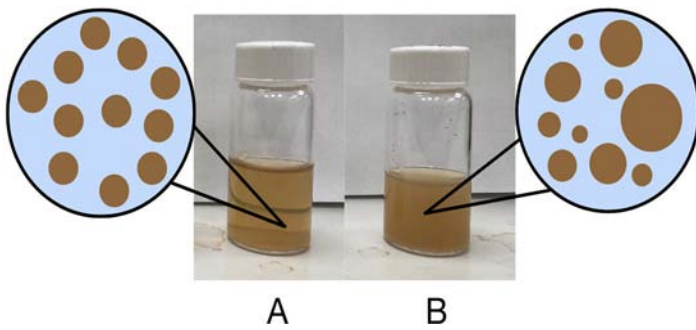


Figure 1. Two dispersion systems with differing PDIs: A) Monodisperse, B) Polydisperse.

are relatively similar in size. Samples with a high PDI are considered polydisperse and are usually characteristic of a broken emulsion (Figure 1). Finally, zeta potential is a measurement of the net surface charge of nanoparticles. A high magnitude of zeta potential is desired for a stable colloidal system as the particles are more likely to repel each other and stay dispersed within the medium (Danaei et al., 2018; Tekin et al., 2020; Wiącek & Chibowski, 1999).

Methods and Materials

To develop and analyze the emulsion systems, the methodology outlined by Ho et al. (2018) was followed. A 5.8% w/w

kava solution was created using 1.45 g of raw kava root powder and 23.55 g of milli-Q water. The solution was mixed for 10 min utilizing a stirring plate. This was done for each of the 9 samples. Following, each sample was given a respective emulsifier, either polysorbate 20 or polysorbate 80, at varying concentrations (0.5–1.5% w/w). The control samples were given no emulsifiers. The samples were then homogenized using a high-shear mixer at 3000 rpm for 30 s. Each sample was then centrifuged 3 times at 2632 x g for 15 min. Between each round of centrifuging, the supernatant containing the kavalactones was separated from the pellet. The final samples were then measured periodically over the course of 10-days utilizing a Malvern Zetasizer Nano ZS. This instrument measured the particle diameter (Z-average), PDI, and zeta potential. Finally, the data was analyzed utilizing the two-way and one-way analysis of variance (ANOVA) and post-hoc test (Tukey-Kramer method) with values considered significant at $P < 0.05$.

Results

Polysorbate 80 at higher-concentrations reduced particle size the most and managed to retain this particle size throughout the 10-day period (Figure 2). However, the PDI for all polysorbate 80 samples was quite high indicating that there was a significant amount of size variance in these samples, which is characteristic of a broken emulsion system. Comparatively, for the polysorbate 20 samples, all samples maintained a

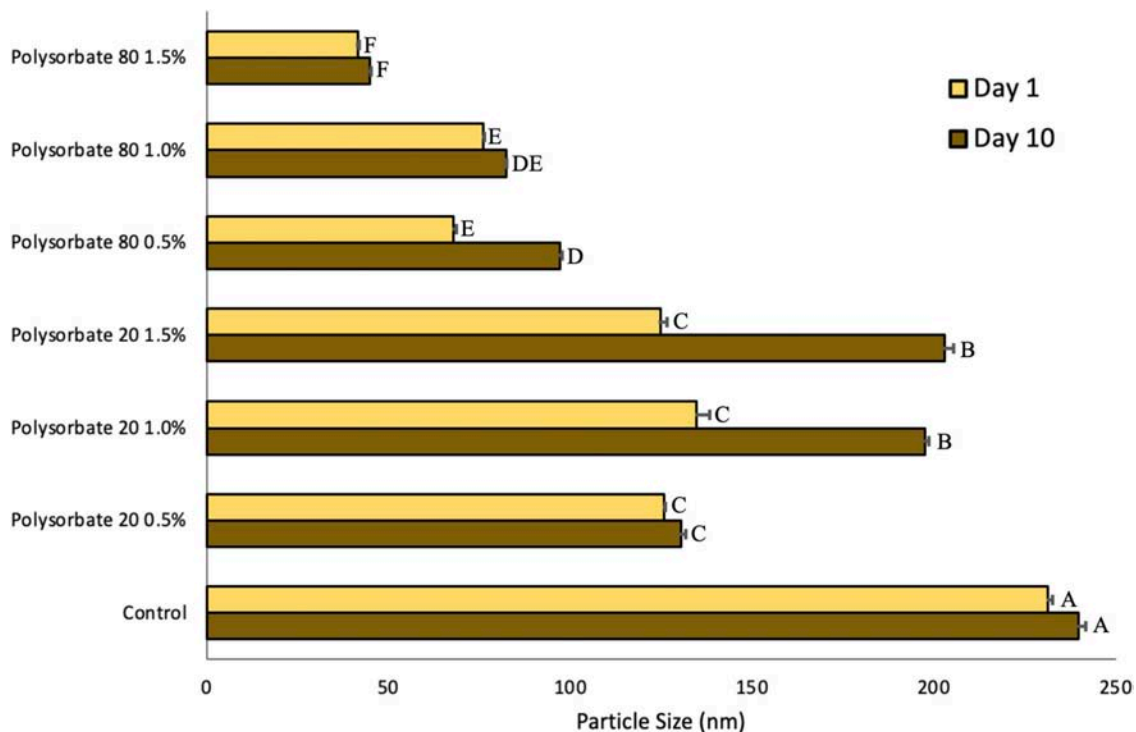


Figure 2. Change in particle size of the various kava dispersions. Mean \pm SD ($n \geq 3$) * $p < 0.05$. Means with the same letter are not significantly different from each other.

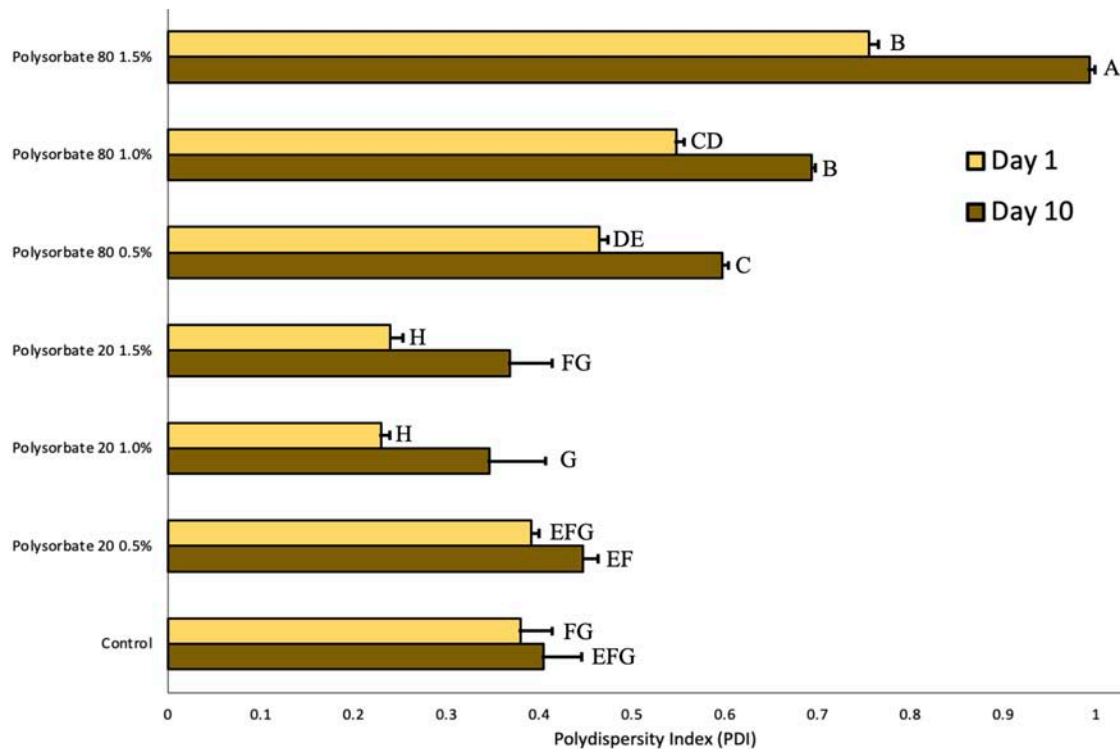


Figure 3. Change in PDI of the various kava dispersions. Mean \pm SD ($n \geq 3$) * $p < 0.05$. Means with the same letter are not significantly different from each other.

PDI between 0.3–0.45, which is considered stable for a dispersal system (Figure 3). On the first day, the average particle diameter for this sample was 130.4 nm which was 107.8 nm smaller than the controls. There were no significant differences in terms of particle size for the different concentrations of the polysorbate 20 on day 1. However, only the 0.5% w/w concentration maintained this particle size over the 10-day period (Figure 2). In terms of zeta potential, both the polysorbate 20 and 80 samples had a zeta potential that was on average 15 mv less than the controls in terms of magnitude (Figure 4). Even though this is not ideal for emulsion stability, the difference in zeta potential was not enough to cause significant differences in aggregation (Wiącek & Chibowski, 1999).

Discussion

As demonstrated by the results of the study, polysorbate 80 at all concentrations failed to develop a stable kava emulsion system. Even though this sample was 196.7 nm smaller than the controls on average and managed to reduce particle size the most, the PDI for this sample was too high to be considered a stable monodisperse colloidal system (Figures 2 & 3). On day one, the PDI for this sample varied from 0.46–0.76 which indicates a high magnitude of particle size variance within the system. This variance was likely caused by unemulsified kavalactones and free polysorbate 80 which broke from

the emulsion system (Danaei et al., 2018). The bulky size of polysorbate 80 could have potentially caused this issue as steric hindrance likely prevented proper emulsification of the kavalactones (Chou et al., 2005). Over time, these unemulsified molecules likely caused aggregation of more particles causing the emulsion to break even more (Tekin et al., 2020). Considering that all polysorbate samples exhibited a relatively low magnitude of zeta potential in comparison to the controls, this likely further contributed to the issue as the aggregating particles did not repel each other and continued to clump together (Figure 3). This is not ideal for a bioactive emulsion system intended for consumption, as larger particles that have broken from the emulsion system can impact the delivery and performance of the active ingredients within the emulsion. In addition, this can cause aggregation within the target tissue or along the digestive tract which can cause health complications (Danaei et al., 2018).

For polysorbate 20, all samples maintained a relatively low PDI (0.3–0.45) over the 10-day period which is suitable for emulsion stability (Figure 3). In terms of particle size, there were no significant differences on day 1 for the different concentrations of polysorbate 20. These samples on average had a particle size that was 107.8 nm smaller than the controls at the start of the 10-day period. However, only the 0.5% w/w concentration managed to maintain a particle size that was significantly less than the controls over the 10-day period (Figure 2). This may potentially be due to the

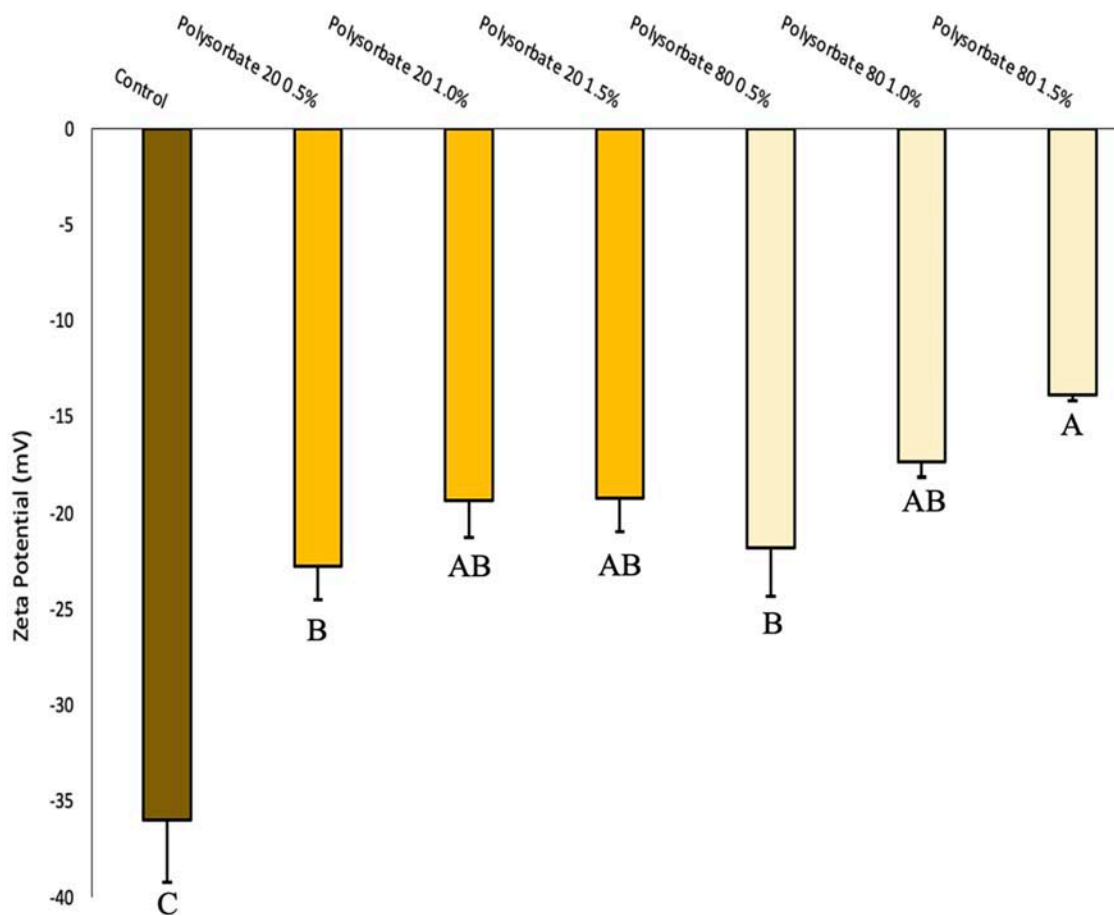


Figure 4. Average zeta potential of the various kava dispersions. Mean \pm SD ($n \geq 3$) * $p < 0.05$. Means with the same letter are not significantly different from each other.

higher-concentration samples having too much surfactant within them. As a result of this, some of the polysorbate 20 did not bind to a kavalactone and remained free within the solution causing aggregation over time (Danaei et al., 2018).

CONCLUSION

Polysorbate 20 at 0.5% w/w concentration managed to produce the most physically stable kava emulsion system. This sample started with a particle size of 125.8 nm and ended with a size of 130.4 nm. By day 10, this sample was 109.3 nm smaller than the controls which is significant in terms of emulsion stability (Figure 2). This sample also managed to maintain a PDI of less than 0.45 over the 10-day period indicating that the particles within the system are quite uniformly sized (Figure 3). Even though the zeta potential for this sample was 13.2 mv smaller than the controls in terms of magnitude, this did not cause any significant aggregation over the 10-days (Figure 4).

After determining that polysorbate 20 at lower concentrations exhibited the most physical stability, further testing with concentrations ranging from 0.1–0.75% w/w will be done to determine the ideal concentration in terms of stability.

Following these tests, the chemical stability of polysorbate 20 will then be analyzed utilizing the methodology outlined by Zhang et al. (2015) in which an in vitro digestive tract will be used to stimulate the varying conditions of the digestive system. If the polysorbate 20 sample retains stability following this chemical analysis, then the emulsion systems developed from this surfactant will likely increase the bioavailability of the kavalactones within the human body (Danaei et al., 2018). As a result of this, the efficacy of using kava for the treatment of anxiety and insomnia would increase, thus making kava an effective alternative to benzodiazepines (Pescitelli et al., 2010; Sarris et al., 2012).

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