ABSTRACT SUMMARY
Celecoxib loaded polyactide-co-glycolide (PLGA) nanoparticles (NP) were developed through a solvent evaporation process using varying concentrations of didodecyl(dimethylammonium bromide (DMAB) or poly vinyl alcohol (PVA) as stabilizer (0.1%, 0.25%, 0.5% or 1% w/v). Characterization of stabilizer free formulation showed zeta potential, particle size, and percent drug entrapment levels of 2.08 ± 0.13 mV, 240.73 ± 9.25 nm, and 9.94 ± 0.01, respectively. The use of 0.1%, 0.25% and 0.5% w/v DMAB stabilizer concentrations significantly increased zeta potential and drug entrapment while significantly reducing particle size. Peak zeta potential and particle size reduction was achieved at 0.5% and 0.25% DMAB concentrations (20.03 ± 0.84 mV and 99.97 ± 3.27 nm, respectively). The inclusion of 1% w/v DMAB concentration resulted in a peak percentage of drug entrapment (61.07 ± 0.06) and a significantly increased particle size (905.01 ± 0.02 nm) when compared to stabilizer free formulations. All PVA formulations exhibited significant alterations in zeta potential values, with a peak zeta potential value reached at 0.25% w/v PVA stabilizer concentration (-6.09 ± 1.39 mV). In comparison to stabilizer free formulation, percent drug entrapment was significantly reduced in all PVA formulations with the exception of 0.1% w/v formulation (46.19 ± 0.16). Our results show effective utilization of concentration based, stabilizer formulations in the synthesis of celecoxib loaded PLGA-NPs.

INTRODUCTION
Celecoxib is a cyclooxygenase-2-selective inhibitor used for treatment of osteoarthritis and rheumatoid arthritis [1]. Although celecoxib is considered to be safer in regards to gastrointestinal tolerability, adverse cardiovascular and renal effects are still major issues of concern. As such, a variety of delivery systems have been developed to help alleviate unwanted side effects.

PLGA is a polymer commonly used in pharmaceutical research. It is an FDA approved biodegradable and biocompatible polymer that undergoes degradation to nontoxic end products lactic acid and glycolic acid [2]. PLGA has been commonly used for celecoxib loaded NP formulation over the years with varying results [3, 4]. However, orally delivered celecoxib loaded NPs have yet to be formulated with the inclusion of known NP stabilizers. The aim of this research was to develop a PLGA-NP carrier of celecoxib utilizing DMAB or PVA as stabilizer. Using a solvent evaporation technique, PLGA based celecoxib loaded NPs were characterized based on size, stability and entrapment efficiency. Effects of differing stabilizer concentrations on NP characterization were also examined.

EXPERIMENTAL METHODS
NPs were prepared by a solvent evaporation technique. Briefly, 20 mg of celecoxib and 50 mg of PLGA were placed in 3 mL ethyl acetate and stirred at 750 rpm for 30 minutes. Afterwards, 30 mg of lecithin was dissolved in the polymer solution then 500 µL acetone was added as co-solvent. Varying concentrations of DMAB or PVA stabilizer (0.1%, 0.25%, 0.5%, or 1% w/v) were placed within 6 mL of high-performance liquid chromatography grade water and stirred at 750 rpm until fully dissolved. Organic phase was added to aqueous phase in a drop wise manner under moderate stirring followed by sonication for 5 minutes at 20 KHz. After sonication, solutions were stirred at 750 rpm for 1 hour to ensure complete organic phase evaporation. After which, each solution was centrifuged at 12,000 rpm and supernatant was collected.

For characterization studies, NP size was measured in triplicate by dynamic light scattering using a NICOMP particle sizer (Particle Sizing Systems, Port Richy, FL, USA). Zeta potential was estimated on the basis of electrophoretic mobility under an electrical field. Entrapment was measured by adding 100 µL NP solution to 300 µL acetonitrile. Solutions were then vortex mixed for 30 seconds. After controlling for blank NPs, 100 µL of drug loaded NP solutions was analyzed under ultraviolet–visible spectroscopy at 260 nm. Celecoxib stock solution dissolved in acetonitrile was used to construct a standard calibration curve (10,000 – 1,000,000 ng/mL) prior to NP solution analysis.

All experiments were performed in triplicate. NP characteristic data is represented as mean ± standard deviation (SD). A Student’s t-test was used to compare each NP formulation with plain formulation.

RESULTS AND DISCUSSION
Celecoxib encapsulated PLGA-NPs were developed to include either DMAB or PVA as stabilizer. The incorporation of celecoxib into DMAB or PVA formulated NPs led to positively and negatively charged NPs, respectively. Based on its cationic nature, DMAB formulated NPs reached a peak zeta potential of 20.03 ± 0.84 mV at 0.5% concentration while the more anionic PVA formulated NPs reached a peak zeta potential of -6.09 ± 1.39 mV with 0.25% concentration. All formulations containing stabilizer resulted in significant alterations in zeta potential when compared to stabilizer free formulations (plain formulation) (Fig. 1).
Further characteristic studies showed a significant particle size reduction in formulations incorporating 0.1%, 0.25%, and 0.5% DMAB when compared to plain formulation (Fig. 2). The use of 1% DMAB resulted in a significant increase in particle size when compared to plain formulation. The highest decrease in particle size was obtained using 0.25% DMAB concentration, which reached a nanometric range of 99.97 ± 3.27 nm.

When compared to plain formulations, use of PVA stabilizers at 0.1% concentration did not result in any significant difference in particle size (p > 0.77). Particle size parameters of 0.25%, 0.5% and 1% PVA formulations were not measurable due to reduced drug/solution concentrations.

The amount of celecoxib entrapped (1.99 ±0.01 mg) and percent of the drug entrapped (9.94 ± 0.01) in stabilizer free formulations were compared to DMAB and PVA formulations. Entrapment studies showed significant alterations in NP entrapment with all stabilizer formulations when compared to plain formulation (Table 1). Increases in celecoxib entrapment were seen in all DMAB formulations and 0.1% PVA formulation with a maximum percent entrapment of 61.07 ± 0.06 reached with 1% DMAB formulations.

Interestingly, as PVA concentrations increased above 0.1% concentrations, a significant reduction in drug entrapment was noted (Table 1). Reduced entrapment when utilizing PVA as stabilizer can be attributed to the hydrophilic nature of PVA [5]. As PVA stabilizer concentrations increase, NP hydrophilicity increases. Increased hydrophilic NP properties could lead to solubilization of formed NPs into the aqueous medium following organic phase evaporation. A rise in NP solubility in aqueous medium would result in net loss of overall drug entrapment following end stage centrifugation resulting in marked reductions in final solution drug concentrations.

**CONCLUSION**

A solvent-evaporation technique was used to developed and characterize celecoxib loaded PLGA-NPs using DMAB or PVA as stabilizer. NPs were characterized based on size, stability and percent of drug entrapped. Our study shows that the use of DMAB as stabilizer resulted in sufficient size and stability alterations with moderate increases in drug entrapment when compared to plain formulation.

**REFERENCES**


**ACKNOWLEDGMENTS**

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![Table 1. Effects of stabilizer concentrations on celecoxib entrapment.](image1)

<table>
<thead>
<tr>
<th>Stabilizer</th>
<th>Conc. (% w/v)</th>
<th>AE (mg)</th>
<th>% EE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stabilizer free</td>
<td>0</td>
<td>1.99 ± 0.01</td>
<td>9.94 ± 0.01</td>
</tr>
<tr>
<td>DMAB</td>
<td>0.1</td>
<td>3.78 ± 0.01</td>
<td>18.85 ± 0.07*</td>
</tr>
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<td>0.25</td>
<td>9.94 ± 0.08</td>
<td>49.70 ± 0.38*</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>6.16 ± 0.01</td>
<td>30.84 ± 0.04*</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12.22 ± 0.01</td>
<td>61.07 ± 0.06*</td>
<td></td>
</tr>
<tr>
<td>PVA</td>
<td>0.1</td>
<td>9.23 ± 0.03</td>
<td>46.19 ± 0.16*</td>
</tr>
<tr>
<td>0.25</td>
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<tr>
<td>0.5</td>
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</tr>
<tr>
<td>1</td>
<td>0.66 ± 0.03</td>
<td>3.28 ± 0.14*</td>
<td></td>
</tr>
</tbody>
</table>

All values reported as mean ± SD (n = 3). EE is the entrapment efficiency. Amount entrapped (AE) per 20 mg celecoxib. * P < 0.01 compared to plain formulation.

![Figure 1. Zeta potential measurements of DMAB and PVA formulated NPs of celecoxib. Values are expressed as mean ± SD, n = 3. * p< 0.01, significantly different from plain formulation.](image2)

![Figure 2. Particle size analysis of increasing concentrations of DMAB stabilizer compared to formulation without stabilizer (plain formulation). Values are expressed as mean ± SD, n = 3. * P< 0.01, significantly different from plain formulation.](image3)