better precipitate formers. For a system with a defined $[API:IP]_{sat}$, and constant starting API and IP initial concentrations, the fraction of initial API that precipitates increases as the K_s of the complex decreases (FIG. **5**A). If the complex exhibits K_s above a critical value, no solids are formed. These results highlight that IPs with strong counter ion interactions with the API maximize complex formation—which is reflected in the experimental results observed, where IPs with lower acid form pK_a are more effective in forming precipitates, even if the IP itself is less hydrophobic.

[0093] For a system with a specific $[API:IP]_{sat}$ and K_s, the fraction of initial API that precipitates increases as the initial concentrations of API and IP increase, as shown in FIG. **5**B. The following conditions were used to generate FIG. **5**B: 10^{-2} M<$[API]_0$<10^2 M; $[API]_0=[IP]_0$; 10^{-6} M<$[API:IP]_{sat}$ <1 M; and $K_s=1$ M^{-1}. Below a critical starting API and IP initial concentrations, no precipitates are formed. The complex can be driven to nearly complete complexation of API by increasing the IP concentration, which corresponds to Le Chatlier's principle. However, this only occurs if individually the species are soluble. If one species precipitates or micellizes, then adding more of that species does not further drive the reaction. In that case the individual ion concentrations in Eq. 3 would be given by the solubility product of the insoluble solid phase or by the CMC of the micellizing species. In that case an additional set of solubility products and mass balances would have to be added to the balances given by Eqns. 3-12. If there are two solid phases that can be formed, that is, an insoluble ion pair complex (API:IP) and a solid phase of the IP, then NCs can be formed, but the stoichiometry of the core of the NC will not be determined by the stoichiometery of the ion pair, but by the molar ratio of complex and insoluble IP. These results provide a model to guide IP choice and precipitation reaction conditions for NC processing, and the model supports the experimental results observed.

Flash NanoPrecipitation of API:IP Complexes

[0094] The four IP candidates (OA, SDS, DBS, PA) that passed the screening process were used to form NCs with the FNP process. Gentamicin and polymyxin B were precipitated with IPs and dissolved into THF as described above, and mixed with PCL-PEG dissolved in THF to yield an organic THF stream containing both polymeric stabilizer and API:IP complex. This stream was rapidly mixed against water within a confined impingement jet to precipitate API:IP complex in the presence of the amphiphilic PCL-PEG to form NCs. DLS analysis of the resultant samples demonstrated that NCs 50-200 nm with narrow polydispersity were formed (FIGS. **6**A and **6**B, Table 1).

[0095] No particles, only PCL-PEG micelles, were formed when only stabilizer and API, or when only stabilizer and IP, was used in the FNC process, with the exception of OA (Table 1). Large aggregates were formed when only API and IP was included during the FNC process, demonstrating that the inclusion of all three stabilizer, API, and IP components are simultaneously needed for NC formation. These results demonstrate that while water-soluble APIs cannot be formulated into NCs, hydrophobic API:IP complexes can be formed into NCs with the water-precipitation process.

[0096] NC formation using pre-formed API:IP complex is, therefore, demonstrated. However, there are advantages to in situ API:IP NC complex formation, since NC formation could be accomplished in a single step. This single step would simplify processing. That is, although APIs can be pre-formed into hydrophobic API:IP complexes using batch processes prior to FNP, a single continuous processing step that can result in both API complexation and NP formation can simplify production procedures and reduce production costs. To test in situ complexation and NC formation and assess if APIs can be encapsulated in a single continuous step by in situ complexation, polymyxin B dissolved in water was impinged against organic solvent containing IP and PCL-PEG in the FNP process. The IPs previously identified as capable of forming NCs using pre-formed complexes were studied using this method. Out of the IPs assessed, all converted polymyxin B into hydrophobic forms in situ during mixing to yield stable NCs with narrow size distributions (FIG. **6**C). By comparing FIGS. **6**B and **6**C, where FIG. **6**B is for the pre-formed IP, and FIG. **6**C is the in situ ion paired polymyxin, one sees that the NC sizes produced by either process are essentially equivalent. Control experiments, in which PCL-PEG and IP only, or PCL-PEG and polymyxin B only were used resulted in empty micelles and not in NC formation. Control experiments where IP and polymyxin B only were used resulted in the formation of aggregations, together demonstrating that all three components—stabilizer, API, and IP—are required for in situ complexation and NC formation.

[0097] To measure the encapsulation efficiency of polymyxin B, free un-encapsulated drug was separated from that which was encapsulated in NCs through ultrafiltration across a 100 kDa membrane, and characterized with BCA analysis. The encapsulation efficiency is based on the relative ratio of free drug compared to the total amount of drug included in the FNC system.

$$\text{Encapsulation Efficiency} = 1 - \frac{[\text{Polymyxin } B]_{free}}{[\text{Polymyxin } B]_{total}}$$

At a 1:1 IP to API charge ratio, polymyxin B was encapsulated with efficiency greater than 95% efficiency for all IPs tested. At excess IP, high encapsulation efficiency was retained, but at a 0.5:1 IP to API charge ratio, encapsulation efficiency dropped significantly, to ~70% in the case of sodium oleate. These results demonstrate that decreasing the amounts of IP relative to API that is present can cause decreased encapsulation efficiencies, by increasing the relative amount of solubilized API that is not retained in the core by ion pairing.

NC Stability

[0098] NC stability was assessed by measuring the size distributions of the NCs when diluted into a closed volume of water or PBS, all at room temperature. Although characterization of NPs in a closed volume does not capture the sink conditions that would be present in vivo, behavior in closed systems can shed insight on NP properties. When paired with at least one charge equivalent of counterion for the four counterions tested, nanoparticle size remained mostly constant in water, despite minor ripening or swelling. When diluted tenfold into PBS, however, particles exhibited size changes over time, which are driven by ion exchange between the anionic IP and the Cl^- and PO_4^{-3} ions in PBS. The ion exchange releases the bound API, which is soluble in the external phase. In the case of pamoic acid IPs,