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The present study investigates the effect of the preparation method (four methods) and formulation additives (propylene glycol (PG) and cholesterol (CH)) on the entrapment efficiency (EE) of pyridoxine hydrochloride (vitamin B6 (VB6)), representing hydrophilic water-soluble low permeable vitamins, in unilamellar liposomes. The main aim is to compare determined EE with predicted values generated using a web-published, computational model. Results showed that among the different preparation methods, modified film hydration showed significantly higher EE (p < 0.05). With regard to formulation additives, PG (5% w/v) produced smaller vesicles size with narrow size distribution. Agreement between determined and model-generated EE values was more evident in formulae with narrow size distribution (polydispersity index (PdI) below 0.23). Formulae containing PG showed slightly higher determined than predicted EE values indicating vitamin-phospholipid bilayer interaction. Meanwhile, agreement between determined and predicted EE was limited to VB6-to-phospholipid ratio below (1.2:2). The comparison provided further insight into the usefulness of the prediction model factors affecting agreement between determined and predicted EE data.