**Fortification of Skimmed Milk with Vitamin B12 with Formation of Double Emulsions using Ultrasound**

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**Statement of Problem:** Vitamins and antioxidants are necessary elements for a healthy body that helps body cell’s growth as well as maintaining a proper mental statue. According to the latest WHO records, Vitamin B12 is going to be epidemic around the globe and it’s probably the most prevalent malnutrition in the future. B12 is a vital co-enzyme for cell growth and diminishing the B12 level in the body leads to weakness, diarrhea, weight reduction, anemia and fatigue. Consumption of enriched food supplements is the best method to prevent health risks caused by diminishing B12 in the body. Moreover, B12 is an indicator of the water phase in emulsion therefore it could be used as an indicator of double emulsions in encapsulations of bioactive compounds.  
**Research Purpose:** The current study evaluated variation of PGPR concentration used in oil phase, the formula of primary and secondary emulsion, and emulsion sonication period to determine optimized primary emulsion formula as well as double emulsion optimized formula. Additionally, particle size, polydispersity index and precipitation Index of primary and secondary emulsions as well as the efficiency of encapsulation were determined. Moreover, optimized formulas were evaluated in terms of variation of different characteristics.

**Research Method:** The internal aqueous dispersed phase (W1) was formulated by dissolving 0.001 M phosphate buffer (pH 6.8) containing 0.1 M NaCl to balance the osmotic pressure between aqueous phases, sodium azide (0.02%, w/v) as microbial inhibitor and Vitamin B12 (0.2% w/v) as water-soluble vitamin. Oil phase was consisted of sunflower oil containing (6-10%) PGPR as a lipophilic surfactant. For preparing W1/O emulsion, premixed the W1 phase (10-30%) with the O phase was done through a magnetic stirrer (RET basic, Germany) at 1000 rpm for 10 min. Then the mixture was emulsified by using a prob ultrasound homogenizer (Parsonic, Daneshpajouh Ltd., Iran) operating at a frequency of 20 KHz with amplitude of 70% (3 s on; 3 s off) for various time (4-6 min) inside a 15 mL test tube to form W1/O emulsion. The primary emulsions were used immediately to prepare the double emulsions or maintained at room temperature for further analysis. Double emulsions were formulated by adding W1/O emulsion (5-25 % w/w) into skimmed milk as an external aqueous phase (W2). For this purpose, the mentioned prob ultrasound homogenizer (Parsonic, Daneshpajouh Ltd., Iran, 20 KHz, 3mm, microtip ultrasonic horn) was applied at a frequency of 20 KHz and amplitude of 70% (3 s on; 3 s off) for various time (1-5 min) inside a 15 mL test tube. The double emulsions were stored at room temperature and analyzed immediately. (Regulating the composition of W1/O and W1/O/W2 emulsions was done.)

**Results and Conclusion:** Since Vitamin B12 is an important bioavailable component for human health, efficient encapsulation of vitamin B12 is investigated in the current study. The main purpose of this investigation was optimization of primary and double emulsion containing vitamin B12 in internal water phase. Formula of primary and double emulsion was determined according to diameter of droplets, Polydispersity index, diameter of double emulsion droplets, and efficiency of encapsulated double emulsion using RSM method. Encapsulation of vitamin B12 was reported to be 97 percent. Finally, results showed that optimized criteria for primary emulsions were 9% PGPR, 20% inner water phase in primary emulsion and 6 minutes sonication time as well as 12% primary emulsion in outer water phase and 1 minute sonication time and 10% primary emulsion in outer water phase and 2 minutes sonication of double emulsion.

**Keywords:** Ultrasound, Double emulsion, Vitamin B12, Skim milk, Encapsulation