



Polymeric nanoparticles: influence of polymer, surfactant and composition of manufacturing vehicle on particle size

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ABSTRACT

Polymeric nanoparticles gained great concern as one of most important drug delivery systems in the last few decades. The main aim of study was to formulate polymeric nanoparticles (PNPs) using pH-sensitive polymers via nanoprecipitation method and investigate the influence of different formulation conditions on nature of nanoparticles including polymer type, polymer concentration, surfactant type, surfactant concentration and ratio of organic: aqueous phases. Mean size of nanoparticles was found to be directly proportional to polymer concentration, but Eudragit S100[®] nanoparticles were smaller than HydroxyPropyl Methylcellulose (HPMC) phthalate HP55 nanoparticles. Also, surfactant concentration significantly affect nanoparticle size as when surfactant concentration increased, nanoparticle size decrease until certain concentration above which no further size reduction was detected. However, Nanoparticles prepared by using Tween 80[®] were smaller than these prepared by Poloxamer 407[®]. Nanoparticles size was found to reversibly proportional to organic: aqueous phase ratio. These results suggest that nanoprecipitation is an efficient method for preparation of polymeric nanoparticles and mean size of produced is significantly affected by nanoparticles formulation conditions.

Key Words: Nanopercipitation; Eudragit S100; HPMC phthalate HP55; Tween 80, Poloxamer 407.

INTRODUCTION

There is significant interest in recent years in the field of developing biodegradable nanoparticles which can be used as a drug/gene delivery system [1 - 5]. In recent years, polymer–nanoparticle composite materials have attracted the interest of a number of researchers, due to their synergistic and hybrid properties derived from several components. Whether in solution or in bulk, these materials offer unique mechanical [6], electrical [7], optical [7,8] and thermal properties [6,9]. Polymeric nanoparticles can be defined as colloidal particles with size ranging between 10 and 1000 nm and these particles can be prepared by using either natural or artificial polymers [10]. The term “polymeric nanoparticle” includes both nanospheres and nanocapsules. Nanospheres are defined as a polymeric matrix in which the drug is uniformly dispersed (typically as a solid solution in polymer), while in the case of nanocapsules the drug present in the matrix core (either as a solid solution or a solution in oil) which is surrounded by a polymeric membrane [11].

Advantages of polymeric nanoparticles include:[12,13] (1) Stability of volatile pharmaceutical agents can be increased, easily and cheaply prepared in large quantities by a multitude of methods, (2) they offer a significant improvement over conventional routes of administration (both oral and intravenous) in terms of efficiency and effectiveness, (3) they considered the ideal candidates for vaccines delivery, targeted delivery of antibiotics and cancer therapy due to ability of modification of drug release pattern, (4) high concentration of therapeutic agent can be delivered to target site, (5) Polymeric nanoparticles can be easily incorporated into tissue engineering or other activities related to drug delivery.

pH-sensitive polymers are polyelectrolytes that bear in their structure weak acidic or basic groups that either accept or release protons in response to changes in environmental pH. These materials have been used to protect acid labile drugs from degradation by the effect of acidic environment or gastric enzymes, to reduce irritation of gastric

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mucosa caused by some drugs, and to achieve target-specific drug delivery by delivering drugs selectively to the site of absorption [14, 15]. Mechanism of action of enteric coating materials mainly depends on their chemical structure where, enteric coating materials are polymers, which contain acid groups. In the acidic environment of the stomach the acid groups are nonionised, and the coating material is insoluble. Rapid dissolution and drug release is achieved in the upper intestine as a function of pH change in the environment. The material dissolves as a result of ionization of polymer acid groups at higher pH [14]. Enteric coating is a barrier applied to oral medication that controls the location in the digestive tract where it is absorbed.

The advantages of pH-sensitive nanoparticles over conventional nanoparticles include: (a) most carriers have been used as enteric-coating materials for a long time, and their safety has been approved. (b) The carriers dissolve rapidly at specific pH and specific sites, which result in quick drug release and high drug concentration gradient. The phenomenon is helpful for the drug absorption. (c) At the dissolution pH because the nanoparticles turn from solid state to hydro gel state, the bio-adhesion of the carrier to mucosa becomes high at specific fragment, which can facilitate the absorption compared with conventional nanoparticles. (d) The pH sensitive nanoparticles can improve the drug stability more effectively. The term nanoprecipitation refers to quite a simple processing method for the fabrication of polymeric nanoparticles where the nanoprecipitation system consists of three basic components: the polymer (synthetic, semi synthetic or natural), the polymer solvent and the non-solvent.

Nanoprecipitation is also called solvent displacement method or interfacial precipitation method [16-21]. It involves the precipitation of a preformed polymer from an organic phase and the diffusion of the organic solvent in the aqueous medium either in the presence or absence of a surfactant [21-24]. Nanoprecipitation method has some advantages over other methods that used for preparation of nanoparticles which include: (1) the use of the solvents like (Acetone/Ethanol) which are considered less toxic than water-immiscible solvents like dichloromethane and chloroform, (2) nanoparticles are formed spontaneously with high shear, (3) Further purification is not required because of the surfactant and solvent.

The aim of our study was preparation of polymeric nanoparticles via nanoprecipitation method and determine effects of various formulation factors including polymer type and concentration,

surfactant type and concentration and ratio of organic: aqueous phase.

MATERIALS AND METHODS

Materials: Eudragit® S100 was a generous gift from Heinrich's Commercial Agency. HPMC phthalate HP55 was supplied from Shin-Etsu Chemical Company (Chiyoda, Tokyo, Japan). Acetone was supplied from El Nasr for chemical pharmaceutical (ADWIC) company (Qalyub, Egypt). Tween 80 was supplied from Kolb distribution Ltd. (Hedingen, Switzerland). Poloxamer 407 was supplied from BASF (Cairo, Egypt).

Methods

Preparation of polymeric nanoparticles: For preparation of polymer nanoparticles, Eudragit S100 or HPMC phthalate HP55 were dissolved in acetone to form organic phase with various polymer concentration (0.2, 0.4 and 0.8 gm %), then organic phase was added drop wise to aqueous phase containing (0.5, 1 and 2 % w/v) of surfactant (Tween 80, Poloxamer 407) under magnetic stirring at room temperature with various ratio of organic: aqueous phase (1:2, 1:4 and 1:8) as shown in tables 1 and table 2. Nanoparticles were formed and turned the solution into milky colloidal suspension. Then, acetone was removed by continuing stirring for overnight at room temperature [25].

Particle size analysis: Measurement of the mean particle size of the nanoparticles dispersion was conducted with the use of laser diffraction particle size analyzer (Master seizer Hydro MU 2000S, Malvern MU instruments, UK). The final particle diameter was calculated from the average of at least three measurements.

RESULTS AND DISCUSSION

Effect of polymer concentration and polymer type

Figures (1-3) showed the effect of polymer concentration on size of formulated nanoparticles. When Eudragit S100 and HPMC Phthalate HP55 were increased from 0.2 gm% to 0.8 gm% with Tween 80 concentration of 0.5% w/v and phase ratio of (1:2), particle size was increased from 390 ± 9.4634 to 714 ± 2.0548 and from 434 ± 3.0912 to 863 ± 0.9428 nm respectively.

The same results were obtained with using Poloxamer 407 rather than Tween 80, and size increased from 404 ± 8.6538 to 747 ± 1.6997 and from 598 ± 1.633 to 905 ± 4.0277 nm respectively as shown in figures (4-6) [26, 27]. These above results may be due to that increasing the concentration of

dissolved polymer resulted in increasing organic phase viscosity and reduction of the efficiency of stirring which caused formation of bigger emulsion droplets [28]; it is also attributed to that higher viscosity that is expected to increase polymer-polymer and polymer-solvent interactions. [17, 29] These results were found to agree with the results of both Sergio Galindo R et al [30] who prepared nanoparticles of Eudragit L100-55 using nanoprecipitation method to determine effect of polymer concentration on nanoparticle size using different organic solvents and found that in all cases, increasing polymer concentration in organic phase resulted in increasing mean particle size, and David Quintanar G et al. [31] who used emulsion-diffusion method to prepare Eudragit E nanoparticles using Eudragit E/ethyl acetate/PVAL system and cellulose acetate phthalate (CAP) nanoparticles using cellulose acetate phthalate/2-butanone/Poloxamer 407 system and in two systems it was found that there is a transition between micro and nanoparticles depending on polymer concentration in internal organic phase where, as polymer concentration increased, size of produced particles significantly increased. On other hand, these results are disagreeing with those reported in Iman Saad A et al. [32] who prepared poly- ϵ -caprolactone nanoparticles by solvent displacement method and investigated the effect of polymer concentration on particle size; and found that increasing polymer concentration from (0.5 to 0.8% w/v) at surfactant concentration (0.5% w/v) resulted in increasing particle size while, at the same surfactant concentration and increasing polymer concentration to (1% w/v) particle size decreased. Also, increasing polymer concentration from (0.5 to 0.8% w/v) at surfactant concentration (1% w/v) resulted in decreasing particle size while, at the same surfactant concentration and polymer concentration was increased to (1% w/v) particle size increased. These results were attributed to that at low polymer concentration and high surfactant concentration, the solubility of polymer in acetone/water mixture might have increased due to solubilizing effect of the surfactant leading to slower rate of polymer precipitation and formation of larger particles, while at higher polymer concentration the effect of surfactant on solubility was less marked leading to higher precipitation rate and formation of smaller particles.

Higher polymer concentration might also result in increasing viscosity of the organic phase which might decrease the diffusion rate and might lower the rate of Ostwald ripening for the more viscous solutions so smaller particles were produced [33]. Eudragit S100 nanoparticles were smaller than those of HPMC phthalate HP55 although we maintained the same formulation conditions; this

may be due to that polymer molecular weight that influences nanoparticle size as higher molecular weight polymer produces smaller nanoparticles [34]. In our case, Eudragit S100 (150000 g/mole) [35] nanoparticles smaller than HPMC phthalate HP55 (78000 g/mole) [36].

Effect of surfactant concentration and surfactant type:

The effect of surfactant concentration on polymer nanoparticle size was studied using Tween 80 or Poloxamer 407 as a surfactant and with either Eudragit S100 or HPMC Phthalate HP55 as a polymer. The mean nanoparticles size was found to decrease with increasing surfactant concentration from 0.5% to 1% either in the case of Eudragit S100 as shown in figures (7-9) or in the case of HPMC Phthalate HP55 as shown in figures (10-12). This may be due to that increasing surfactant concentrations results in reducing the surface tension and facilitating particle partition. The reduction in the particle size is usually accompanied by a rapid increase in the surface area. Thus, there is an opposition between primary coverage process of newer surfaces and agglomeration of uncovered surfaces. So, rapid coverage of newly formed particle surfaces is a result of increasing surfactant concentration in primary dispersion [37]. But, nanoparticle size increase while increasing surfactant concentration from 1% to 2% and this may be due to that there was an optimum surfactant concentration, above which, any increase in surfactant concentration did not result in further decrease in nanoparticle size due to saturation point [38] but particle size will rather increase due to increase in surfactant adsorption on nanoparticle surface. These results were in full agreement with that obtained from Chander PD et al. [37] who prepared loaded Eudragit L100 using Pluronic[®]F-68 as a surfactant via solvent displacement method and investigated effect of surfactant concentration on particle size and found that, mean particle size was significantly decreased by increasing Pluronic[®]F-68 concentration from 0.25% to 1% w/v.

According to effect of surfactant type, Tween 80 as surfactant resulted in formation of nanoparticles smaller than those obtained by Poloxamer 407 either with using Eudragit S100 or HPMC Phthalate HP55 and this may be attributed to that Tween80 (non- polymeric surfactant) has an advantage over Poloxamer 407 (polymeric surfactant) due to higher adsorption potential than an equal chain length polymer [39]. Our results were in a complete accordance with those of Iman Saad A et al. [32] who prepared poly- ϵ -caprolactone nanoparticles via solvent displacement method using different surfactant types with the same concentration of 0.5% w/v and

it was found that the mean size of nanoparticles prepared by using Tween 80 and Poloxamer 407 (which also known as Pluronic F-127) [40] are 253.6 ± 4.0 and 356.3 ± 5.5 nm respectively.

Effect of organic to aqueous phase ratio: Figures (13-16) showed the effect of phase ratio on Eudragit S100 or HPMC Phthalate nanoparticles with using Tween 80 or Poloxamer 407 as surfactant. It was found that size of nanoparticles was inversely proportional to increasing the ratio of organic to aqueous phase, as nanoparticle size decrease with increasing the ratio of organic phase of the polymer (either Eudragit S100 or HPMC Phthalate HP55) to aqueous phase of surfactant (either Tween 80 or Poloxamer 407). The increase in aqueous phase volume results in decreasing the particle size due to increase of diffusion rate of the water-soluble organic solvent (acetone) in the aqueous phase [41]. Thus, larger particle size was obtained for formulations containing less aqueous phase. The obtained results are in complete settlement with the results of Swarnali Das *et al.* as he prepared loaded Eudragit RL100 nanoparticles for ocular administration using nanoprecipitation method and stated that, increasing aqueous phase volume regarding to organic phase resulted in decreasing nanoparticle size [42] and attributed that increasing diffusion rate of organic solvent (acetone) in aqueous phase. But, these results are contradictory with the results reported in David Quintanar G *et al.* [31] who used emulsion

diffusion method to prepare Eudragit E nanoparticles and investigated the relationship between particle size and % w/v of Eudragit E in organic phase for batches prepared with different internal (organic): external (aqueous) volume ratios (1:2, 1:3, 1:4) at a fixed concentration of stabilizer (PVAL 5% w/v); and found that there is insignificant difference was observed between the slopes representing the mean size of prepared batches.

CONCLUSION

Formulation factors affecting mean size of nanoparticles prepared by nanoprecipitation (solvent displacement) method were investigated. It was found that polymer concentration and polymer type have significant effect on nanoparticle size. Besides, type and concentration of surfactant affect nanoparticle size till certain limit. In addition, organic/aqueous phase ratio is reversibly proportional to nanoparticle size.

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Table 1: structure of formulas prepared either in the case of ES100 or HPMC phthalate HP55 with concentrations of (0.2, 0.4 and 0.8 gm %) and using Tween 80 as surfactant with various concentrations (0.5, 1 and 2%) and different organic: aqueous phase ratio (1:2, 1:4 and 1:8).

Organic : aqueous phase ratio	Polymer type	Polymer conc. (gm %)	Surfactant	Surfactant conc. (W/V %)	Organic : aqueous phase ratio	Polymer type	Polymer conc. (gm %)	Surfactant	Surfactant conc. (W/V %)
1:02 1:04 1:08	Eudragit S100	0.2 gm%	Tween 80	1%	1:02 1:04 1:08	HPMC Phthalate HP55	0.2 gm%	Tween 80	1%
1:02 1:04 1:08		0.4 gm%			1:02 1:04 1:08		0.4 gm%		
1:02 1:04 1:08		0.8 gm%			1:02 1:04 1:08		0.8 gm%		
1:02 1:04 1:08		0.2 gm%		2%	1:02 1:04 1:08		0.2 gm%		
1:02 1:04 1:08		0.4 gm%			1:02 1:04 1:08		0.4 gm%		
1:02 1:04 1:08		0.8 gm%			1:02 1:04 1:08		0.8 gm%		
1:02 1:04 1:08		0.2 gm%		0.50%	1:02 1:04 1:08		0.2 gm%		
1:02 1:04 1:08		0.4 gm%			1:02 1:04 1:08		0.4 gm%		
1:02 1:04 1:08		0.8 gm%			1:02 1:04 1:08		0.8 gm%		

Table 2: structure of formulas prepared either in the case of ES100 or HPMC phthalate HP55 with concentrations of (0.2, 0.4 and 0.8 gm %) and using Poloxamer 407 as surfactant with various concentrations (0.5, 1 and 2%) and different organic: aqueous phase ratio (1:2, 1:4 and 1:8).

Organic : aqueous phase ratio	Polymer type	Polymer Conc. (gm %)	Surfactant	Surfactant Conc. (W/V %)	Organic : aqueous phase ratio	Polymer type	Polymer Conc. (gm %)	Surfactant	Surfactant Conc. (W/V %)	
1:02 1:04 1:08	HPMC Phthalate HP55	0.2 gm%	Poloxamer 407	1%	1:02 1:04 1:08	Eudragit S100	0.2 gm%	Poloxamer 407	1%	
1:02 1:04 1:08		0.4 gm%			1:02 1:02 1:08		0.4 gm%			
1:02 1:04 1:08		0.8 gm%			1:02 1:04 1:08		0.8 gm%			
1:02 1:04 1:08		0.2 gm%			1:02 1:04 1:08		0.2 gm%			
1:02 1:04 1:08		0.4 gm%			2%		1:02 1:04 1:08			0.4 gm%
1:02 1:04 1:08		0.8 gm%			1:02 1:04 1:08		0.8 gm%			
1:02 1:04 1:08		0.2 gm%		0.50%	1:02 1:04 1:08		0.2 gm%			
1:02 1:04 1:08		0.4 gm%			1:02 1:04 1:08		0.4 gm%			
1:02 1:04 1:08		0.8 gm%			1:02 1:04 1:08		0.8 gm%			

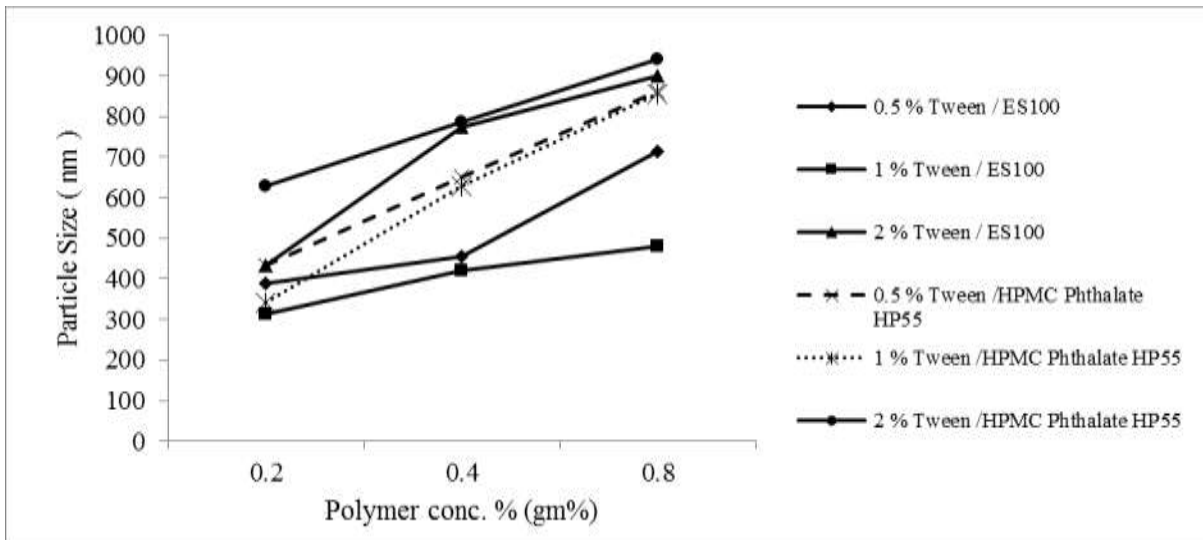


Figure 1: Showing effect of polymer type and polymer concentration on particle size of nanoparticles prepared using Tween 80 as surfactant inorganic: aqueous phase ratio (1:2)

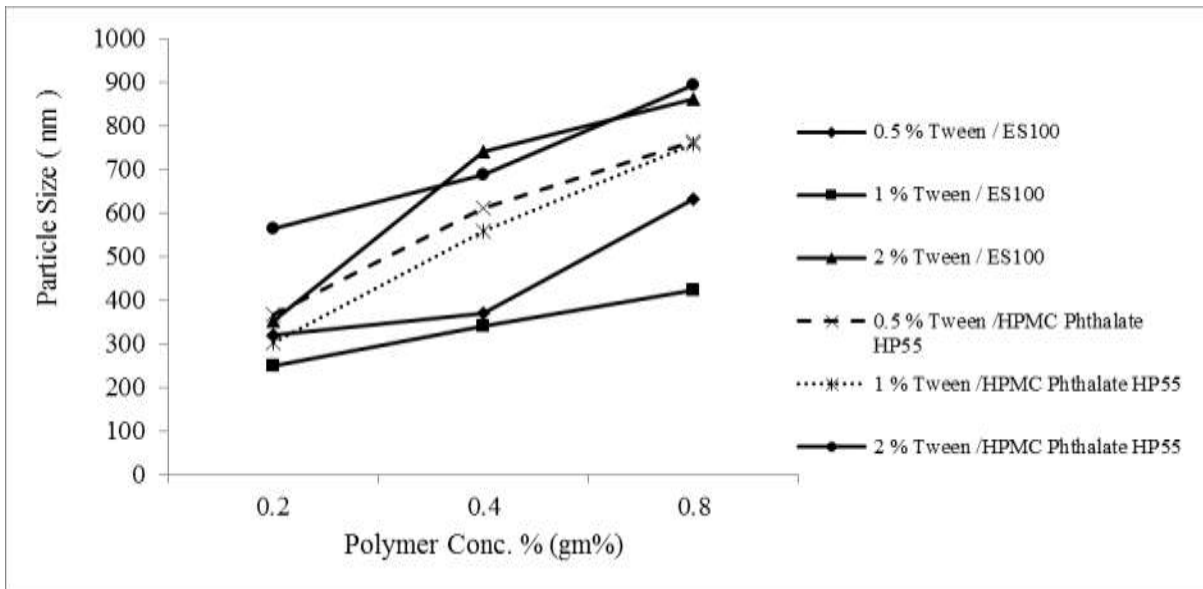


Figure 2: Showing effect of polymer type and polymer concentration on particle size of nanoparticles prepared using Tween 80 as surfactant inorganic: aqueous phase ratio (1:4)

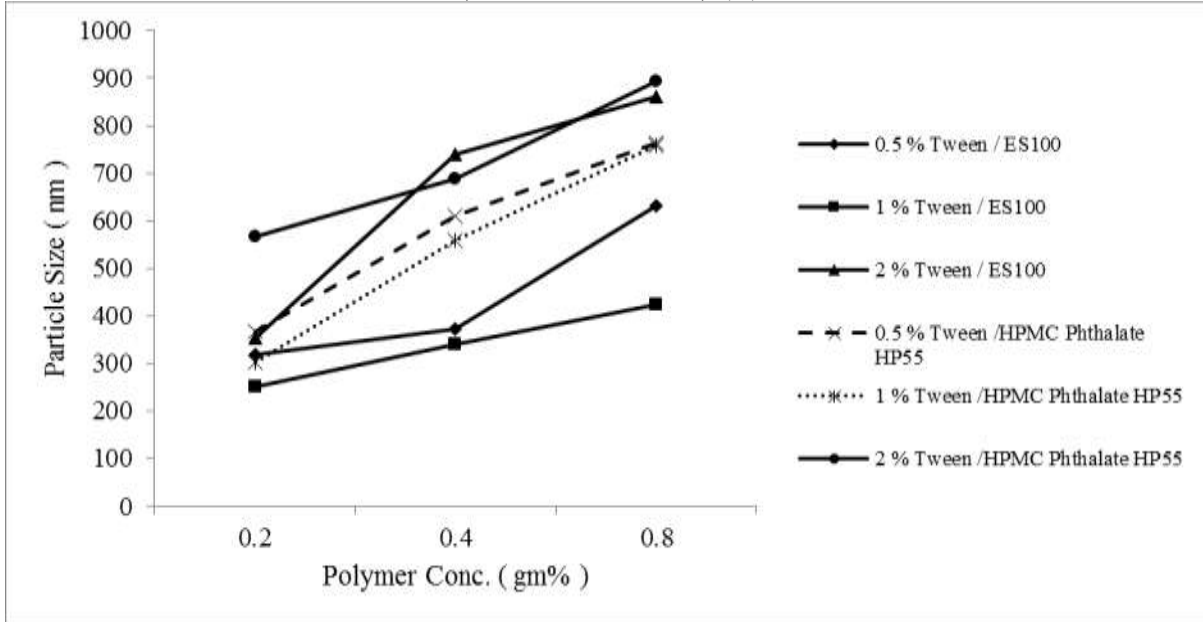


Figure 3: Showing effect of polymer type and polymer concentration using Tween 80 as surfactant and organic phase: aqueous phase ratio (1:8)

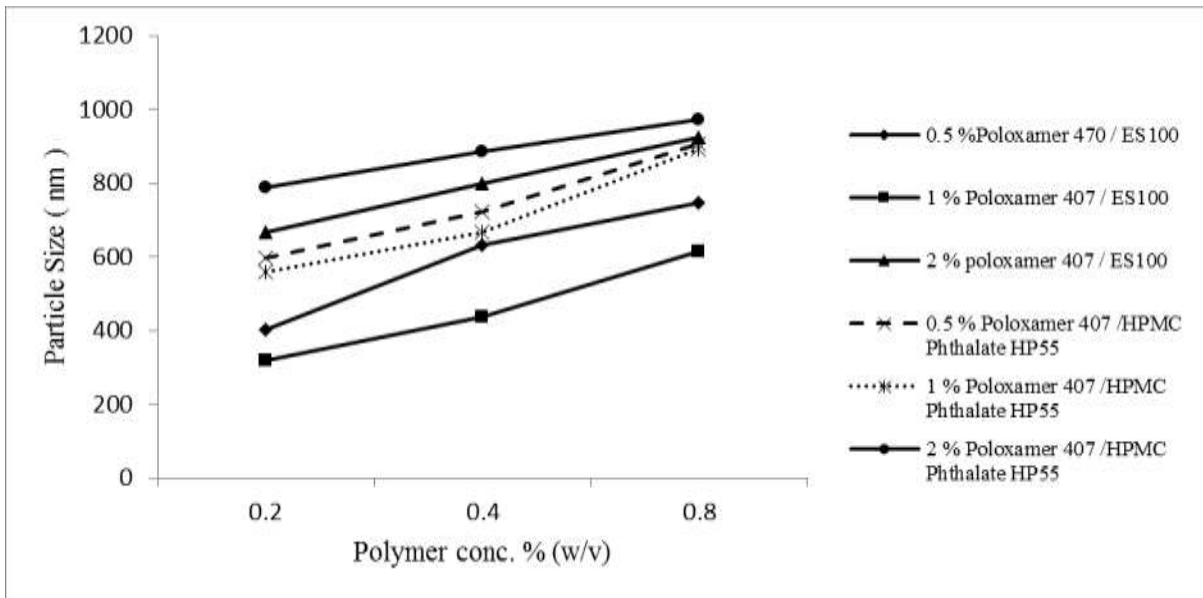


Figure 4: Showing effect of polymer type and polymer concentration using Poloxamer 407 as surfactant and organic phase: aqueous phase ratio (1:2)

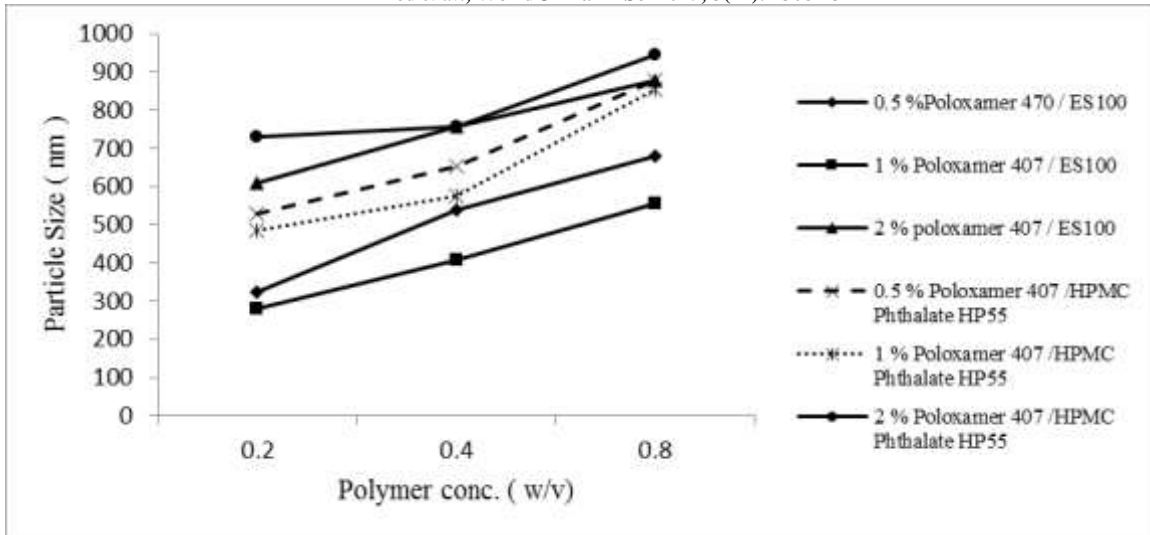


Figure 5: Showing effect of polymer type and polymer concentration using Poloxamer 407 as surfactant and organic phase: aqueous phase ratio (1:4)

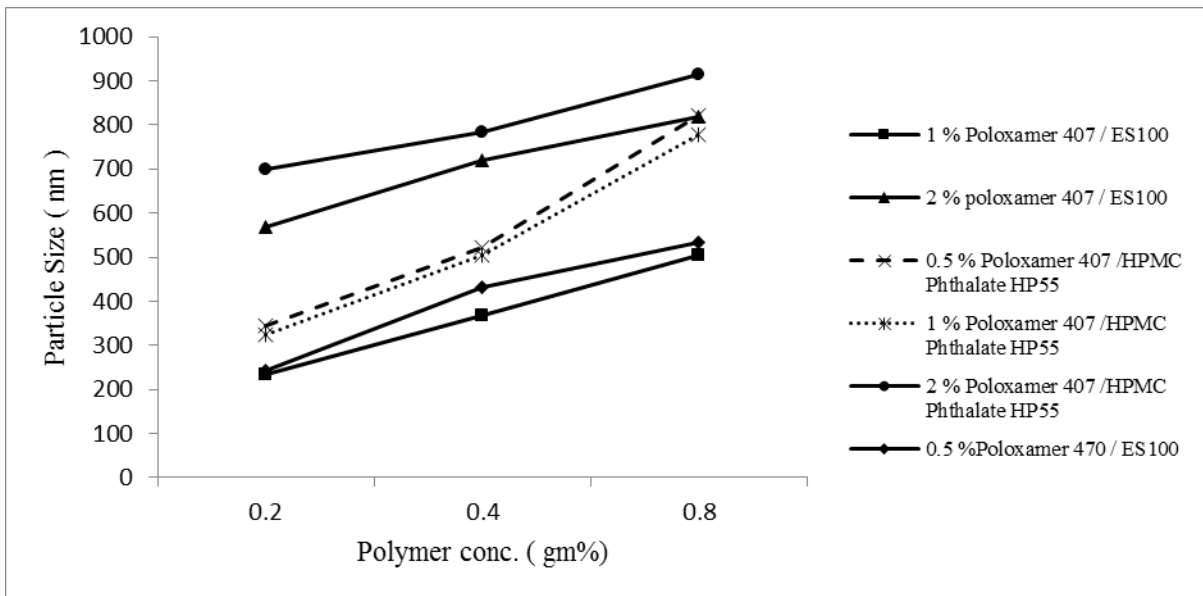


Figure 6: Showing effect of polymer type and polymer concentration using Poloxamer 407 as surfactant and organic phase: aqueous phase ratio (1:8)

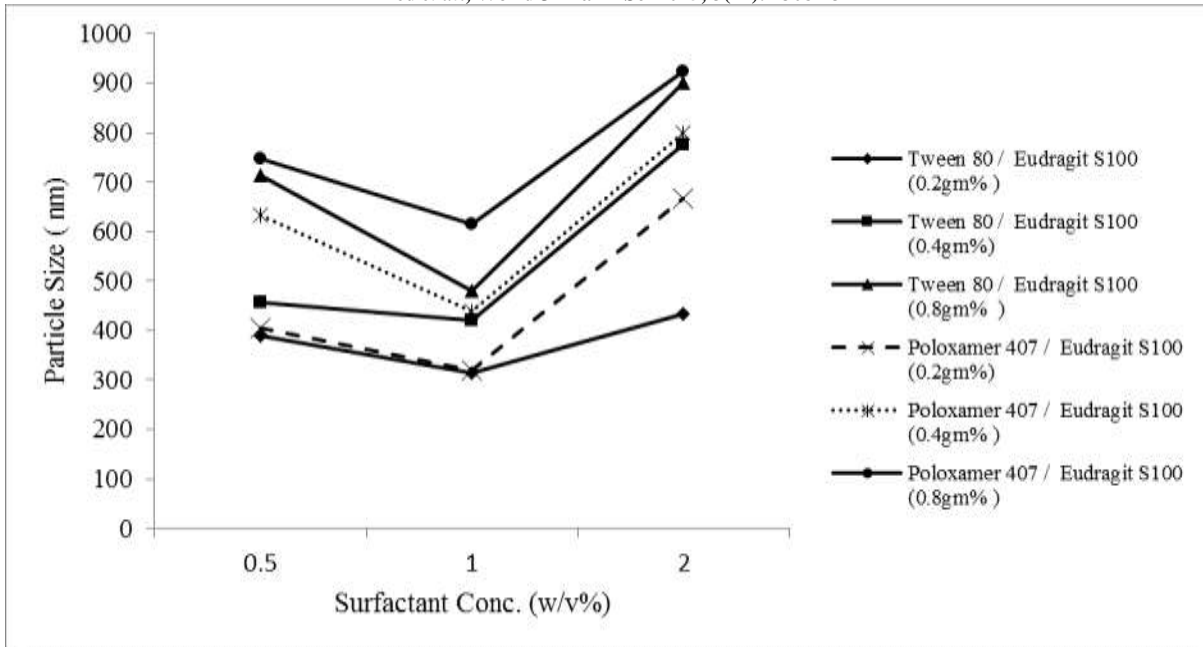


Figure 7 : Showing effect of surfactant type and surfactant concentration using Eudragit S100 as polymer and organic phase: aqueous phase ratio (1:2)

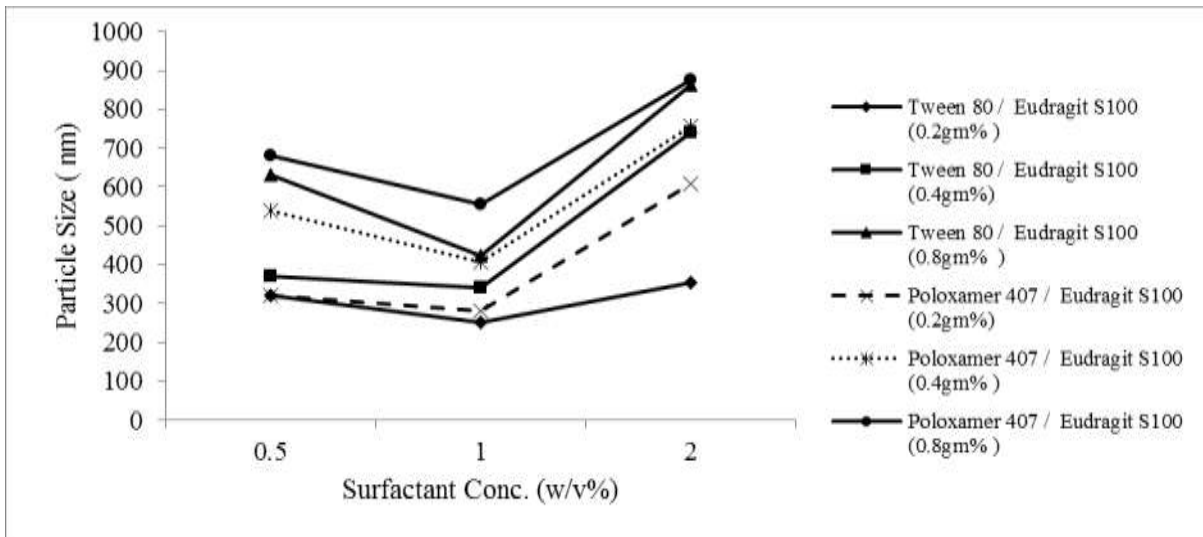


Figure 8: Showing effect of surfactant type and surfactant concentration using Eudragit S100 as polymer and organic phase: aqueous phase ratio (1:4)

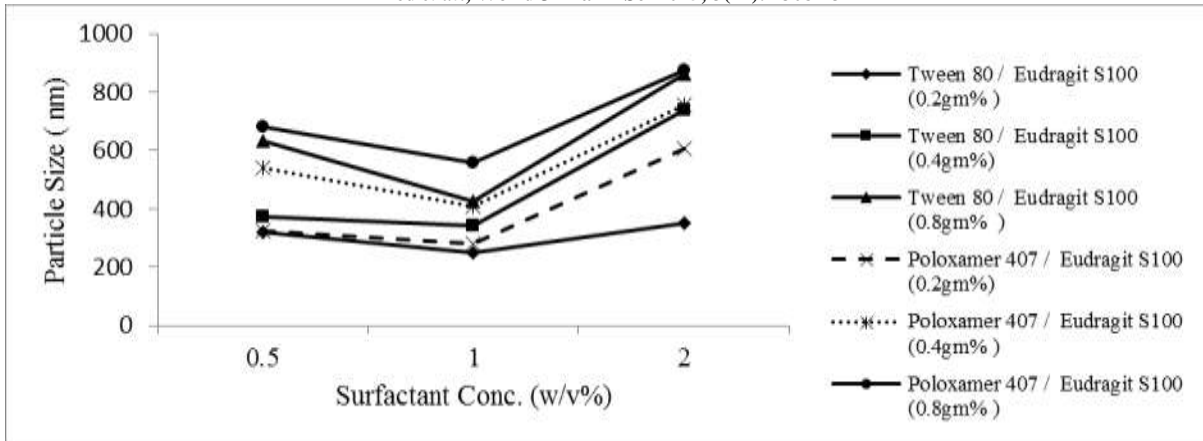


Figure 9: Showing effect of surfactant type and surfactant concentration using Eudragit S100 as polymer and organic phase: aqueous phase ratio (1:8)

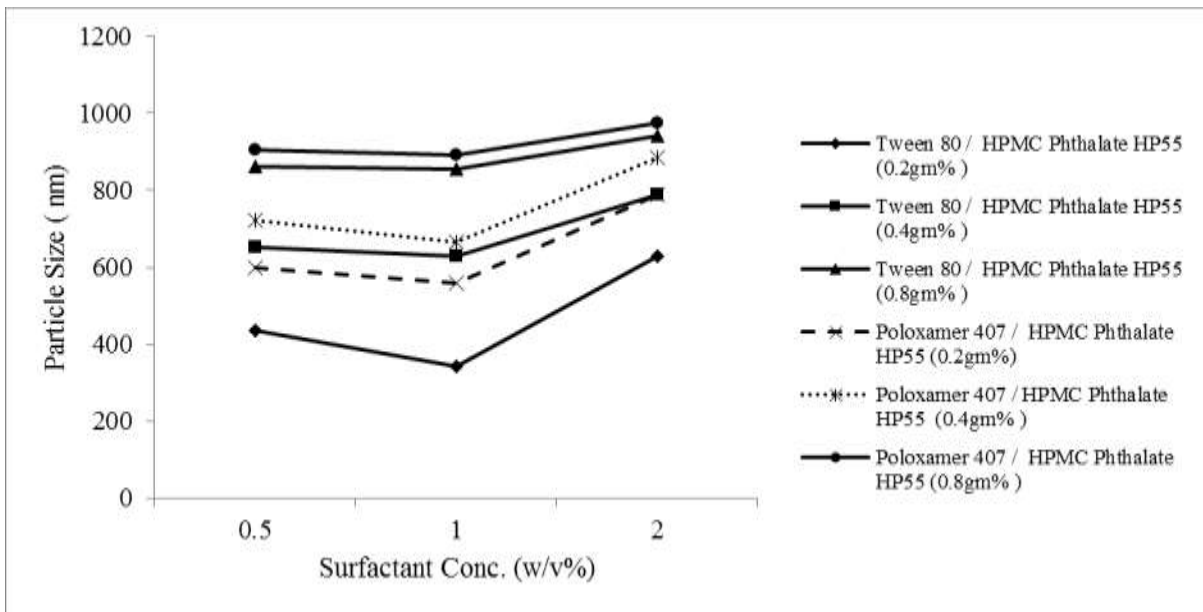


Figure 10: Showing effect of surfactant type and surfactant concentration with HPMC phthalate HP55 and organic phase: aqueous phase ratio (1:2)

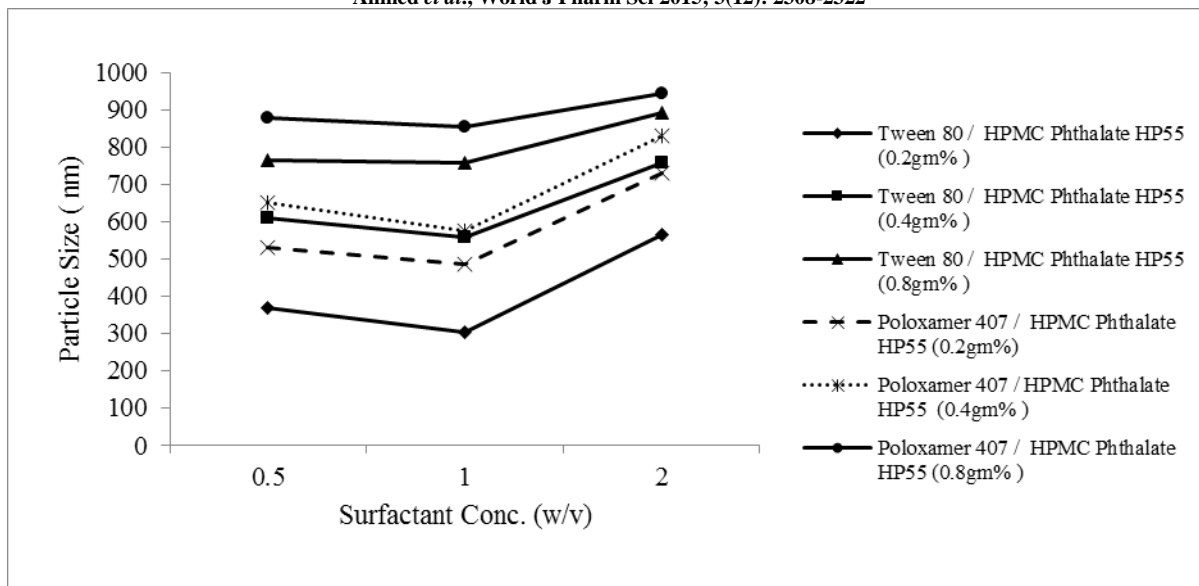


Figure 11: Showing effect of surfactant type and surfactant concentration with HPMC phthalate HP55 and organic phase: aqueous phase ratio (1:4)

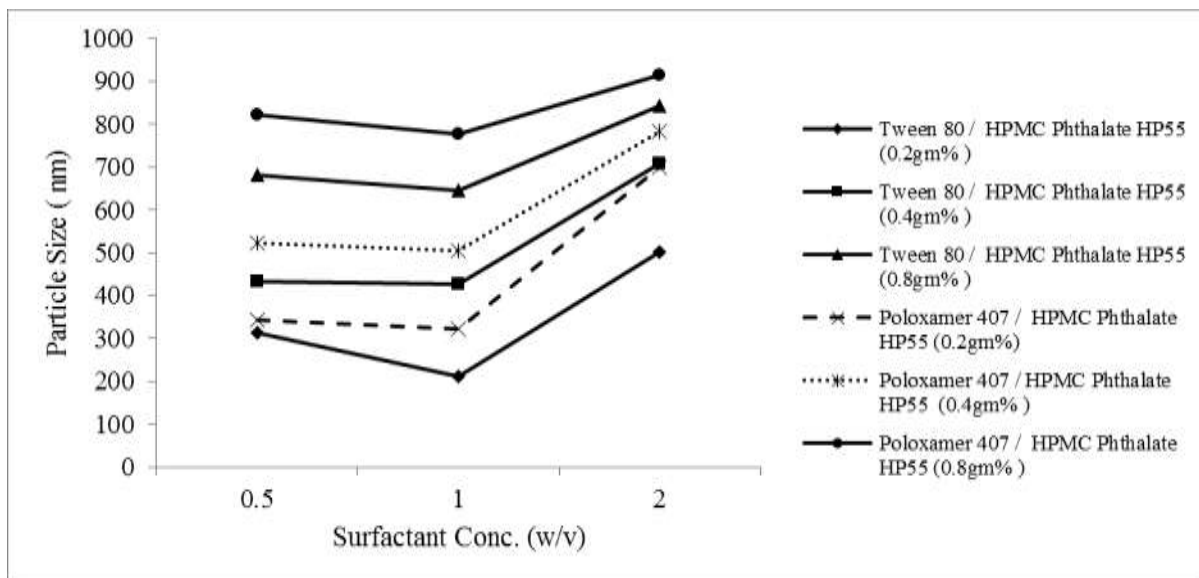


Figure 12: Showing effect of surfactant type and surfactant concentration with HPMC phthalate HP55 and organic phase: aqueous phase ratio (1:8)

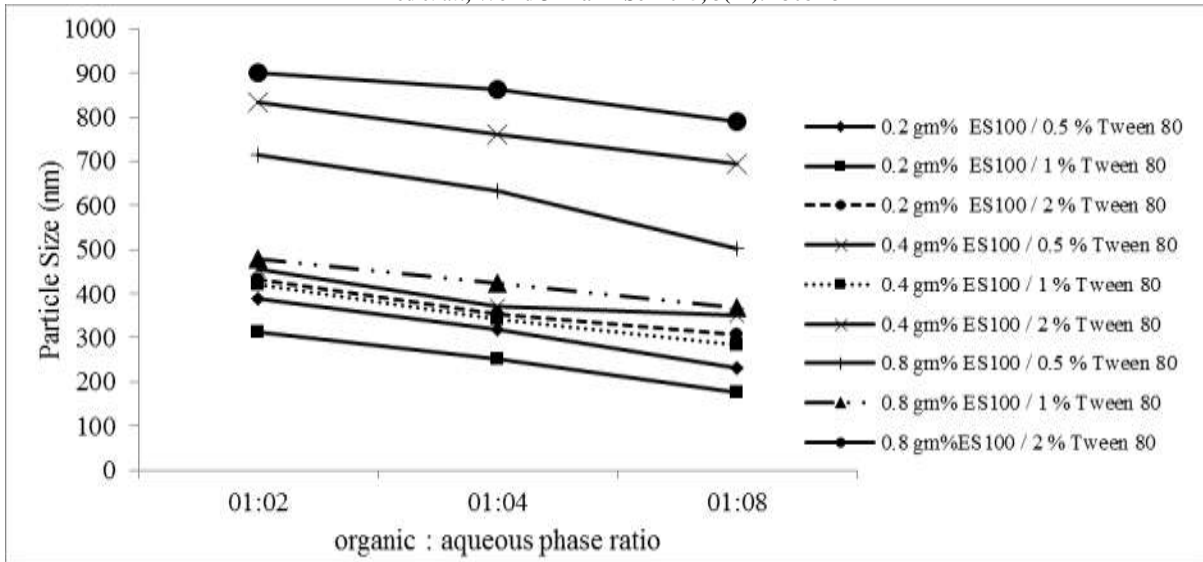


Figure 13: Showing effect of organic phase to aqueous phase ratio using Eudragit S100 with Tween 80

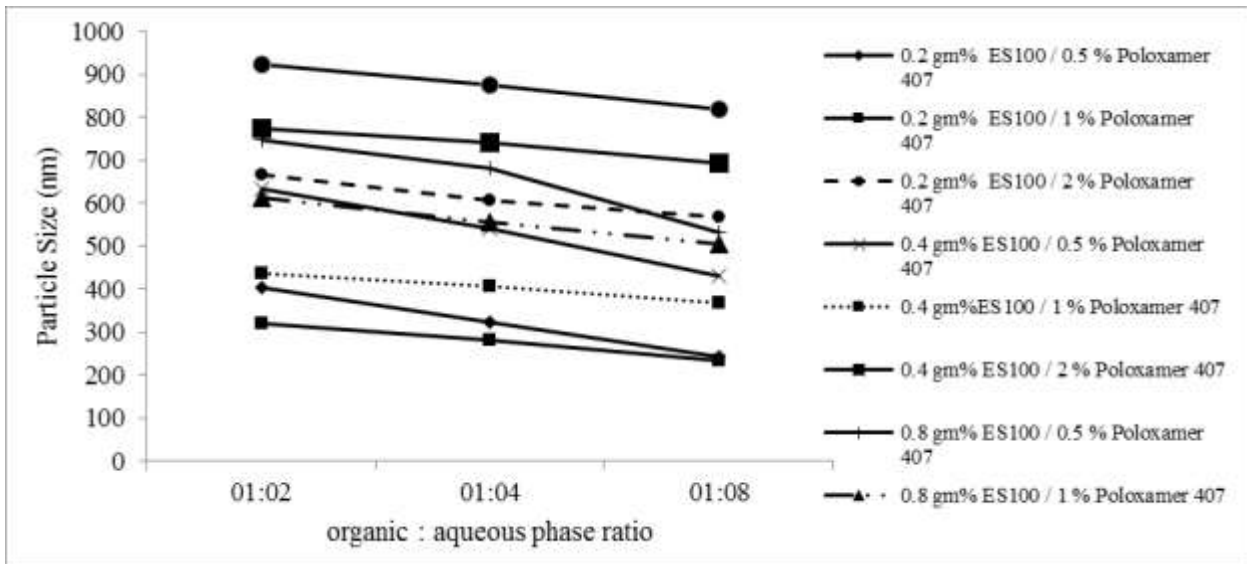


Figure 14: Showing of organic phase to aqueous phase ratio using Eudragit S100 with Poloxamer 407

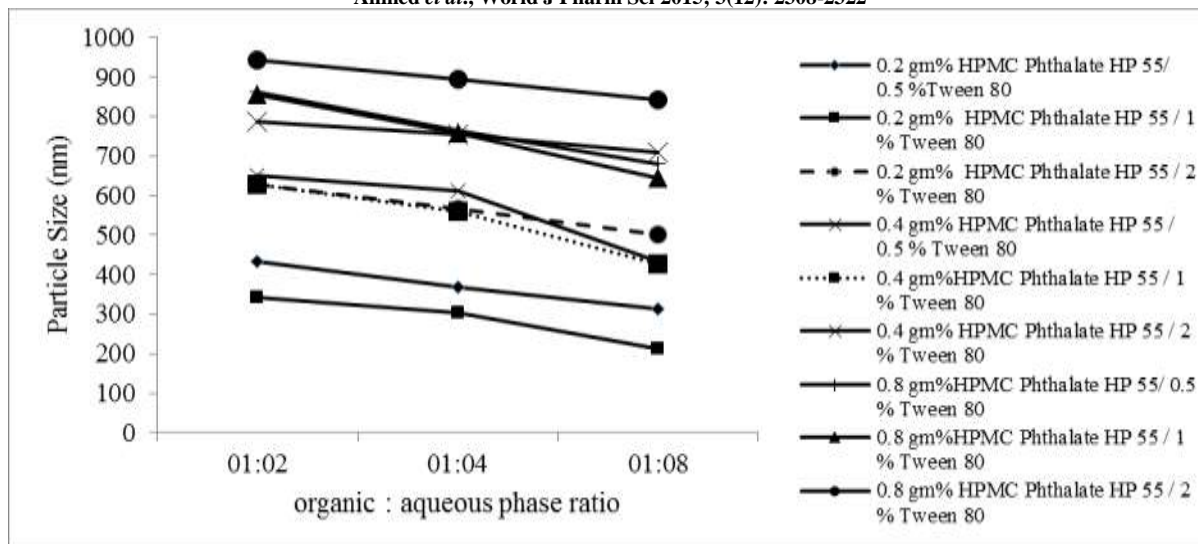


Figure 15: Showing effect of organic phase to aqueous phase ratio using HPMC Phthalate HP55 with Tween 80

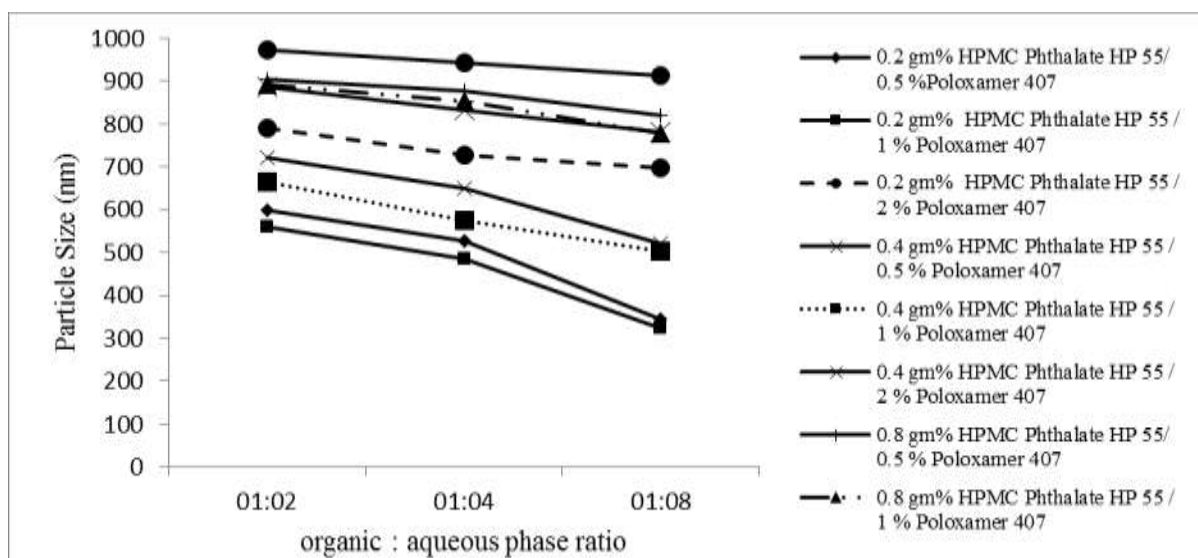


Figure 16: S Response of *Portulaca Oleracea* L. plants to various fertilizers ratios on growth, yield and chemical composition under Egyptian conditions

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