



# Investigation of the properties of encapsulated hydrophilic and hydrophobic drugs in whey protein microparticles

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## ABSTRACT

In this study, the amoxicillin (AMX) and rifampicin (RIF) were loaded into the whey protein (WP) to form the hydrophilic and hydrophobic drugs loaded whey protein microparticles. Scanning electron microscopy (SEM) images demonstrated that AMX addition increased the particle size, but RIF addition decreased the dimensions of the particles. The Fourier-transformed infrared spectroscopy (FTIR) results showed that both drugs were successfully loaded into the protein. AMX and RIF were ultimately released from the WP microparticles after 1440 min, according to the drug release test. Two drugs exhibited the same behaviour, but in the first hour, AMX had more release than the RIF.

## 1. Introduction

Nano and microcapsules are promising systems for drug delivery in the treatment of many disease types. Microencapsulation is a process in which solid or liquid droplets are coated with a continuous layer of polymeric material [1]. Proteins are among the most widely used food biopolymers as encapsulating agents of food bioactive compounds and nutraceuticals. They also provide a higher encapsulation efficiency and/or protective effects than other biopolymers as wall materials for electrospraying [2]. The electrospray method consists of a capillary sprayer and an annular electrode. In this method, the liquid flowing out of the capillary sprayer is kept under high electrical potential pushed by the electrical field and dispersed in very fine droplets [3,4]. WPs are one of the important by-products of the cheese industry. They are widely used in foods due to their superior gelling and emulsification properties. They have good emulsification, gelling and film-forming properties. Therefore, WP can be used as an encapsulated agent [5]. In this study, the electrospray technique was used to fabricate the drug-loaded microparticles. WP was used as a matrix material to encapsulate the drugs. AMX and Rifampicin were added to the WP to observe the effect of the hydrophilic and hydrophobic drugs on the properties of the particles. Amoxicillin is a hydrophilic penicillin-type antibiotic. It is often used in children to treat ear infections and chest infections [6]. Rifampicin is a macrocyclic hydrophobic antibiotic with significant activity against mycobacteria and is commonly used in combination with other agents to treat tuberculosis [7].

## 2. Materials & method

### 2.1. Materials

WP concentrate (MW = 14.178 kDa), Rifampicin (MW = 822.94 g/mol), and Amoxicillin (MW = 365.4 g/mol) were purchased from Sigma Aldrich (USA).

### 2.2. Preparation and characterization of the solutions

20% WP was dissolved in the mixture of 20 ml distilled water and ethanol (5%) on the magnetic stirrer with the parameters of 300 rpm and room temperature for half an hour. 5 mg AMX and RIF were weighed and put into the 20 ml 20% WP solution separately. The homogeneous distribution was obtained after 1 h and 12 h for AMX and RIF, respectively.

The density, electrical conductivity, surface tension, viscosity and pH values were measured to determine the physical properties of the prepared solutions. Density values were calculated using a standard bottle. The electrical conductivity was measured using the Cond 3110, Germany. The surface tension was measured using a force tensiometer Sigma 703D, Germany. The viscosity was determined using a DV-E, Brookfield (USA).

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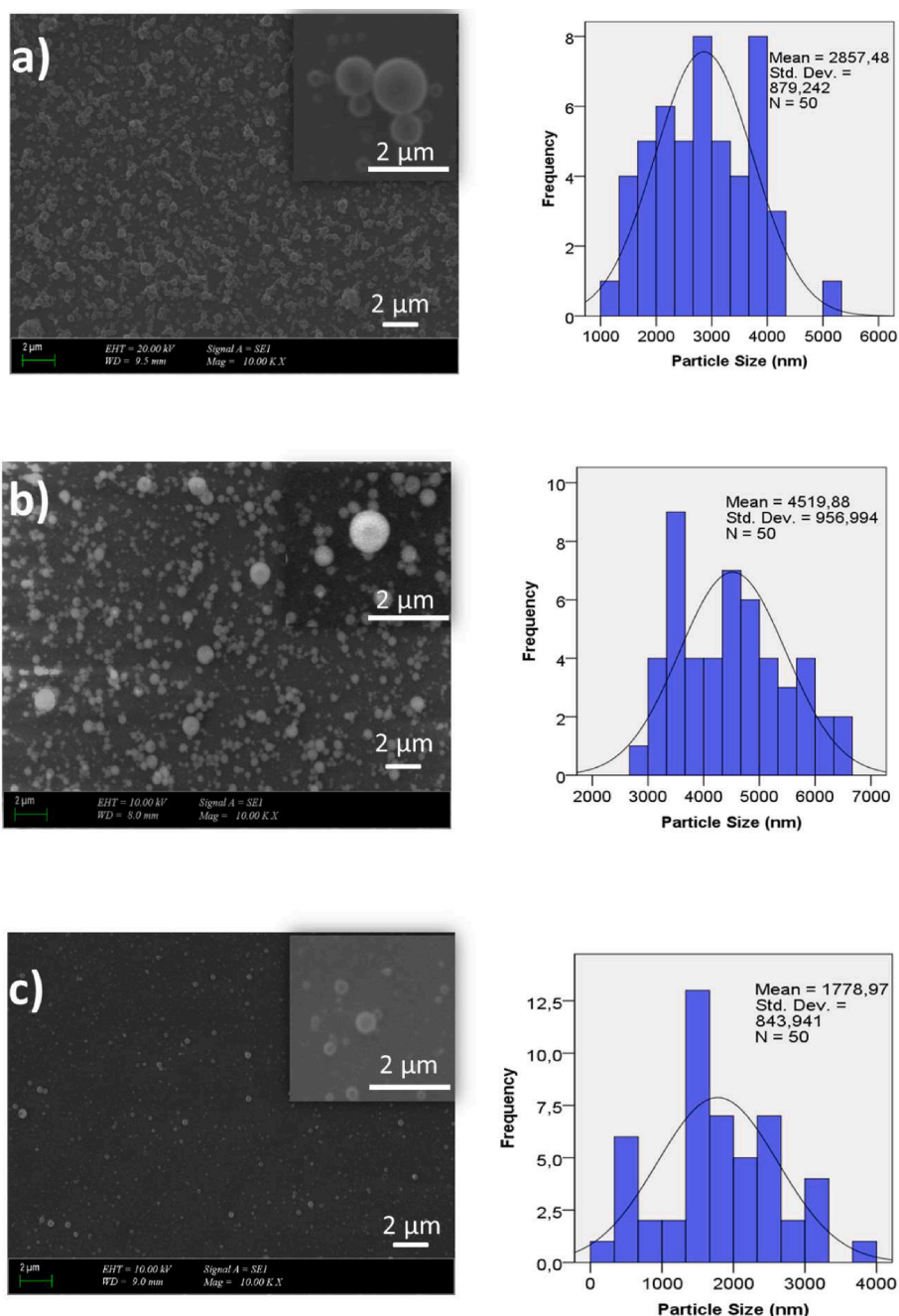
E-mail address: [ulagitu1773@gmail.com](mailto:ulagitu1773@gmail.com) (S. Ulag).

**Table 1**  
Physical properties of the prepared solutions.

Microparticles	Elektrical Conductivity ( $\mu\text{S}/\text{cm}$ )	Density ( $\text{g}/\text{cm}^3$ )	Surface Tension ( $\text{mN}/\text{m}$ )	Viscosity ( $\text{mPa}\cdot\text{s}$ )
20% WP	$2.46 \pm 0.1$	1.07	$38.42 \pm 0.1$	$4799 \pm 127$
20% WP + AMX	$2.46 \pm 0.2$	1.08	$38.17 \pm 0.5$	$3439 \pm 9$
20% WP + RIF	$2.33 \pm 0.3$	1.09	$38.95 \pm 0.5$	$3311 \pm 160$

### 2.3. Electro spray of the microparticles and their characterization parameters

The WP, WP/AMX, and WP/RIF microparticles were fabricated using a laboratory-scale electro spray machine (NS24, InovensoCo, Turkey). A syringe pump (NE-300, USA) was utilized to arrange the flow rate of the solutions and connected to the stainless steel needle (0.3 mm inner and 0.8 mm outer diameters, respectively). The prepared solutions were filled into 10 ml plastic syringe. The flow rate was changed between 0.15 and 0.2 ml/hr. The voltage values were optimized to 22–23 kV, and the distance between the collector and needle was kept constant to 12 cm. The collector was covered with an aluminium sheet, and the microscopic glass slide was put on this aluminium sheet. The morphologies of the particles were examined under SEM. The electro sprayed microparticles on the glass slide were coated with Au for 120 sn before the analysis. The



**Fig. 1.** SEM images of the 20% WP (a), 20% WP/AMX (b), and 20% WP/RIF (c) microparticles.

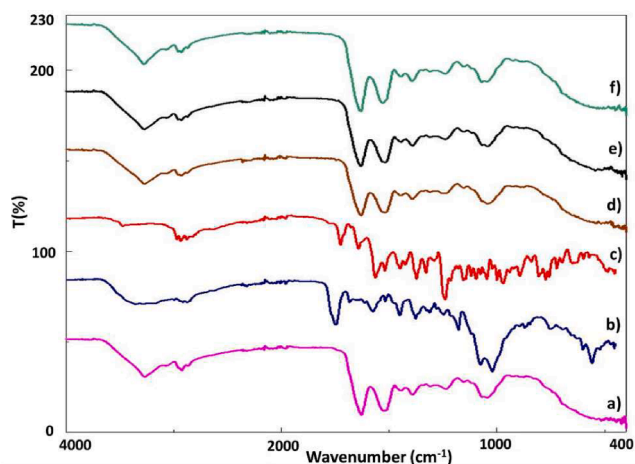


Fig. 2. FTIR spectrums of the pristine WP (a), AMX (b), RIF (c) and 20% WP (d), 20% WP/AMX (e), 20% WP/RIF (f) microparticles.

mean particle sizes of the microparticles were measured using image software (Olympus AnalySIS, USA). FTIR analysis was carried out over the range of  $4000\text{--}400\text{ cm}^{-1}$  with  $4\text{ cm}^{-1}$  resolution. In the drug release test, nearly 5 mg microparticles were weighed and put into the 2 ml eppendorf tubes with 1 ml phosphate-buffered saline (PBS). Then, these tubes were maintained at  $37\text{ }^{\circ}\text{C}$  in a thermal shaker. Then, 1 ml of the PBS solution was taken out after definite time intervals from 15 min to 24 h and analyzed through a UV-visible spectrophotometer (Shimadzu-Japan). During the test, PBS was changed every time with fresh PBS. The standard calibration curves of AMX and RIF were obtained at the wavelength of 190–500 nm to establish the relationship between drug concentration and absorbance.

### 3. Results & discussions

The electrical conductivity values of the solutions are given in Table 1. The results showed that 20% of WP had  $2.46 \pm 0.1\text{ }\mu\text{S/cm}$ . Adding 5 mg AMX into the 20% WP solution, the electrical conductivity value was found the same as the 20% WP solution. On the other hand, the conductivity value decreased to  $2.33 \pm 0.3\text{ }\mu\text{S/cm}$  for 20% WP/RIF solution. The viscosity value of 20% WP was found to be  $4799 \pm 127\text{ mPa.s}$ . By adding AMX and RIF into these solutions, viscosity values decreased to  $3439 \pm 9$  and  $3311 \pm 160\text{ mPa.s}$ , respectively. The surface tension and density values were found nearly the same for all solutions (Table 1). Fig. 1 represents the SEM images of the particles. Fig. 1a belonged to the 20% WP microparticles with their heterogeneous distributions, and the mean particle size was found at  $2857.48 \pm 879\text{ nm}$ . In Fig. 1b, the SEM images of the 20% WP/AMX particles had heterogeneous distributions, and their average particle sizes were found at  $4519.88 \pm 956\text{ nm}$ . The mean particle size of the 20% WP/RIF microparticles was found  $1778.97 \pm 843\text{ nm}$ . It was found that the addition of AMX into the 20% WP increased the size of the particles. However, by adding the RIF into the 20% WP, the size of the particles decreased. This proved that low viscosity causes a small particle size [8]. In addition, it can be said that the hydrophilic and hydrophobic properties of the drugs also affect the sizes of the particles. The FTIR spectrums of the pure WP, AMX, RIF and 20% WP, 20% WP/AMX, and 20% WP/RIF microparticles were given in Fig. 2. In Fig. 2a, the band detected at  $3272.61\text{ cm}^{-1}$  represented the stretching vibrations of  $\text{--OH}$  linked to  $\text{NH}_2$ . Another peak observed at  $2925.48\text{ cm}^{-1}$  showed the  $\text{--CH}_2$  groups. The characteristic band of the primary amide group of proteins ( $\text{--CO--NH}_2$ ) was detected at  $167.63\text{ cm}^{-1}$  and the secondary amide group of proteins ( $\text{--CO--NH}$ ). These two peaks are the characteristic bands of the WP. Other peaks observed at  $1450.21$ ,  $1388.50$ ,  $1236.15$ , and  $1045.23\text{ cm}^{-1}$  represented the  $\text{--C--O}$ ,  $\text{C--C}$ , and  $\text{--C--OH}$  groups [9]. In Fig. 2b, the AMX had main absorption peaks at  $3354.57\text{ cm}^{-1}$  (stretching vibration of the

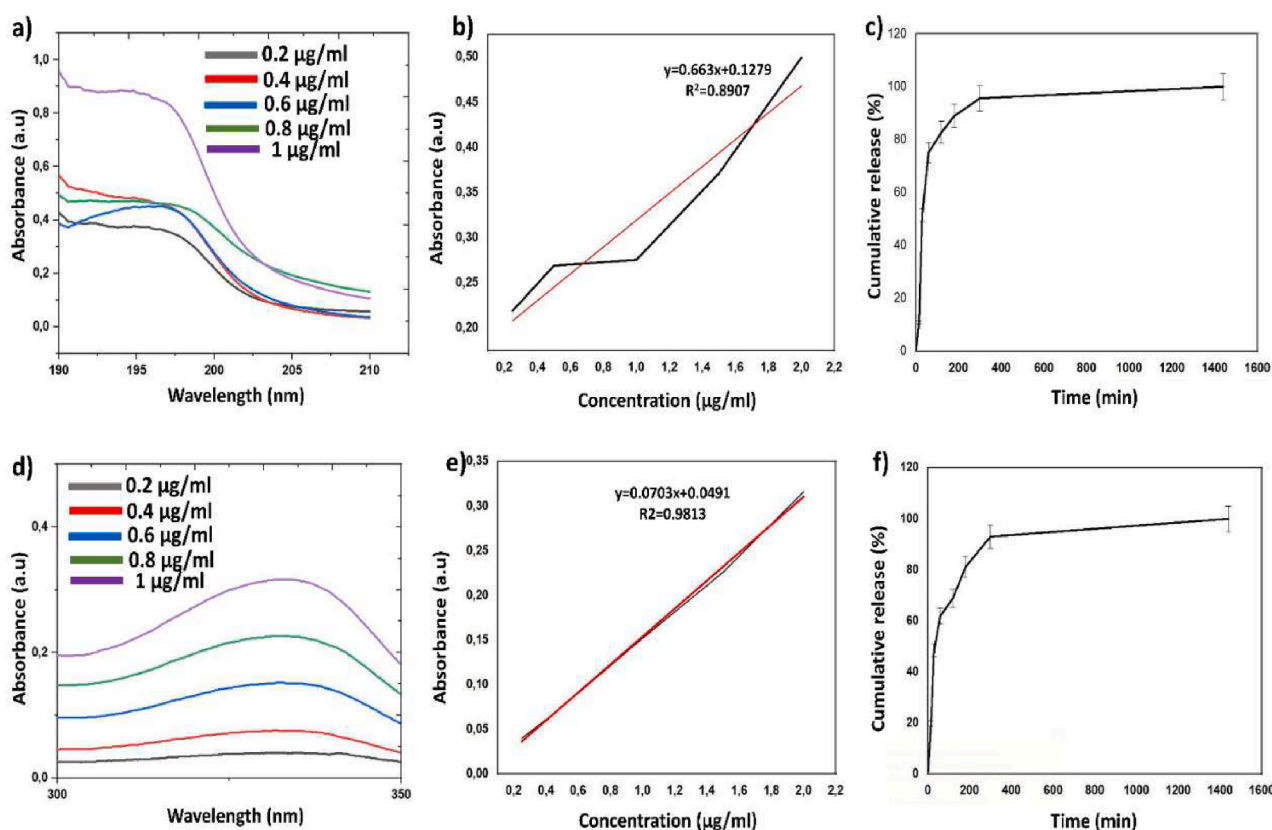


Fig. 3. The calibration curves (a, d), absorbance graphs (b, e) of the AMX and RIF, the cumulative release graphs of the drugs (c, f).

amino group), 2874.38  $\text{cm}^{-1}$  (stretching vibration of the  $\text{CH}_2$ ), 1749.12  $\text{cm}^{-1}$  (carbonyl group), 1683.55  $\text{cm}^{-1}$  (Amide I), 1576.52  $\text{cm}^{-1}$  (Amide II), 1377.89  $\text{cm}^{-1}$  (carboxyl group), and 1247.72  $\text{cm}^{-1}$  (Amide III) [10]. In Fig. 2c, the pristine RIF had main absorption peaks at 3407.60  $\text{cm}^{-1}$  ( $-\text{NH}$ ), 2971.77  $\text{cm}^{-1}$  ( $-\text{OH}$ ), 1724.05  $\text{cm}^{-1}$  (Amide group), 1644.02  $\text{cm}^{-1}$  ( $-\text{C} = \text{O}$ ), and 1563.99  $\text{cm}^{-1}$  ( $\text{C} = \text{C}$ -stretching) [11]. Fig. 2d showed the FTIR spectrum of the 20% WP microparticles, and it had the same peaks as the pure WP. Fig. 2e and 2f belonged to the FTIR spectrums of the 20% WP/AMX and 20% WP/RIF microparticles, respectively. These spectrums were similar to the FTIR spectrum of the 20% WP microparticles except for some shifts. This indicated that drugs were successfully loaded into the protein. The calibration curves of the drugs were determined with five different solutions (0.2–1  $\mu\text{g}/\text{mL}$ ), and the graphs were given in Fig. 3 (a, d). Fig. 3 (b, e) showed the absorbance graphs of the AMX and RIF. Fig. 3c demonstrated the cumulative release graph of the AMX from the WP microparticles, in the first hour, nearly 75.13% of AMX was released, which showed the burst release behaviour of the AMX into the microparticles. After this time, the release became sustainable, and the release finished at 1440 min reached 99.99% cumulative release. The release of RIF from the WP microparticles was given in Fig. 3f. In the first hour, the 61.89% cumulative RIF release was detected, and after this time, the sustainable release was observed until 1440 min. According to the results, it can be said that two drugs reached the cumulative release at the same time, but the release of the AMX from the particles was faster than the release of RIF from the particles in the first hour.

#### 4. Conclusions

This study reported that the AMX and RIF loaded WP microparticles were fabricated successfully with the electrospray method. SEM images demonstrated that hydrophobic drug addition decreased the size of the particles. The release of the drugs into the WP was investigated, and results showed that AMX and RIF showed nearly the same release behaviour but AMX released faster than RIF in the first hour.

*CRedit* authorship contribution statement

**Kudret Irem Deniz:** Methodology, Investigation. **Songul Ulag:**

Conceptualization, Methodology, Investigation. **Oguzhan Gunduz:** Methodology, Investigation.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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