EMULSION MICROPARTICLES OF PROTEIN AND PECTIN FOR DRUG AND NUTRIENT DELIVERY PURPOSES

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Abstract.

This article explores recent advancements in drug delivery systems (DDSs) using pectin polysaccharides (PP) derived from apples, sunflower, and citrus fruits, combined with whey and silk proteins to form stable emulsion particles in an oil-in-water (o/w) system. Optimal conditions for emulsion stability—including biopolymer ratios, pH, and ionic strength—were identified, enhancing pectin chain efficiency and enabling the formation of a robust interfacial layer.

Polyphenolic compounds (PPhCs) encapsulated in these microcapsules, stabilized by sericin protein and apple pectin, demonstrated high antioxidant activity at minimal concentrations, confirming the preservation of their therapeutic properties. Remarkably, emulsions with high PPhC encapsulation maintained significant antioxidant activity even at 100-fold dilution.

These findings contribute to developing biopolymer-based encapsulation technologies with potential applications in pharmaceuticals, food and cosmetics, agriculture, and molecular diagnostics.

Keywords: pectin, lactoglobulins, sericin, drug delivery system, emulsion.

1. Introduction.

Drug delivery systems (DDS) play a crucial role in modern medicine, enhancing the effectiveness and safety of pharmacotherapy by controlling the release rate, timing, and localization of drugs within the human body. One of the primary advantages of DDS is its ability to target specific tissues or organs, thereby reducing side effects and increasing drug concentration in the affected area.

Developing new drug molecules is a time-consuming and costly process that does not always guarantee success. Despite extensive and advanced medical research, only a few drugs have demonstrated good bioavailability in clinical practice [1].

Among dosage forms with improved pharmacokinetic parameters, drugs utilizing controlled-release mechanisms based on micro- and nanotechnology are well known [1-4]. Over the past few decades, nanoparticle drug delivery systems have been extensively explored to achieve high therapeutic drug concentrations at disease sites [2–6]. This growing interest is driven by the broad applications of effective controlled-release DDS across various industries, including pharmaceuticals [5–8], cosmetics [8], food [9], and other sectors [10,11].

The increasing disease burden worldwide, the proven efficacy of controlled-release drug delivery systems in treating various diseases, and the growing research interest in advanced drug delivery systems are driving market growth. The global advanced drug delivery systems

market is projected to register a compound annual growth rate (CAGR) of 5% during the forecast period from 2019 to 2024 [12].

Advanced drug delivery systems are increasingly designed to maintain drug bioavailability in accordance with patient needs and optimize drug delivery rates. Micro- and nanoemulsion encapsulation is a rapidly developing area of nanotechnology focused on producing monodisperse nanocapsules containing biologically active substances [11].

Nanoparticles and microspheres serve as ideal drug delivery systems for both controlled and targeted drug release. Nanoemulsions are biphasic colloidal systems with dispersed-phase droplet sizes in the submicron range (20–1000 nm). They exhibit high thermodynamic stability and transparency due to the presence of one or more amphiphilic surfactants. Nanoemulsions represent the most advanced type of emulsion system, with significant potential in drug delivery applications, including targeted, mucosal, transdermal, and site-specific delivery, as well as diagnostic applications [11-17]. Micro- and nanocapsules can function as intracellular and in vivo delivery vehicles, enabling the simultaneous transport of multiple biomolecules.

Polysaccharide–protein complex coacervates have been among the most studied biopolymer systems for encapsulating various active ingredients over the past few decades. The precise design of microscale and nanoscale capsules for controlled encapsulation and their application-specific properties remains an area of significant research interest [5, 13-20].

The use of biodegradable polymers, globular proteins, and pectin polysaccharides (PP) in the formation of micro- and nanocapsule shells enables time- and location-controlled delivery of active substances [2-20]. Pectin and protein have been widely utilized in food systems, where their interactions determine the physicochemical properties and texture of food and pharmaceutical products. The primary forces involved in forming protein-pectin composites include electrostatic interactions, hydrophobic forces, hydrogen bonding, and van der Waals forces [4,5].

A key challenge in incorporating biologically active components into food products is their poor stability and susceptibility to degradation under environmental conditions such as pH, temperature, and oxidative stress. Recent research has focused on applying delivery systems for the encapsulation of bioactive compounds in the functional food sector. Huang et al. [20] reviewed recent advances (2016–2022) in utilizing pectin for constructing various emerging nanocarriers, including polyelectrolyte nanocomplexes, protein-pectin core-shell nanoparticles, nanoemulsions, nanoliposomes, and nanofibers. These nanocarriers provide enhanced protection, stability, and controlled release of bioactive compounds, with improved efficacy achieved by combining pectin with other biopolymers, such as proteins.

Despite these advancements, several issues related to microemulsion encapsulation remain insufficiently studied, including emulsion stability, drug-loading capacity per unit mass of the carrier, and kinetic parameters of drug release. Our research focuses on developing such systems using biodegradable pectin components and proteins of natural origin for encapsulating poorly soluble drugs [5, 15-17].

The potential of protein-polysaccharide complexes to encapsulate and deliver *biologically active compounds, nutrients*, and drugs has garnered significant attention in the food, cosmetic, and pharmaceutical industries [21-27]. Proteins, due to their amphiphilic nature, act as emulsifiers, whereas polysaccharides, being hydrophilic, function as thickeners and stabilizers. The digestion and absorption of biologically active compounds (BAC) depend on the physical and chemical stability of the emulsion, which is influenced by various factors such as pH, temperature, ionic strength, and the presence of other surfactants and digestive agents [19-22].

Sericin has long been considered a waste product of the textile industry. However, in recent years, silk sericin has found extensive applications in cosmetic products, prompting further research into its medical applications, including biomedical engineering [28-31]. The use of sericin as an emulsifier and binder biopolymer presents numerous beneficial biological properties, including antioxidant and antityrosinase activities. These properties contribute to the development of biomaterials with medicinal benefits, including anti-inflammatory and tumor-inhibiting effects. Additionally, sericin is being explored for use in biomaterials for wound healing, active packaging of food products, and various applications within the food and pharmaceutical industries.

The aim of this study is to develop a comprehensive approach for obtaining highly stable emulsion microcapsules stabilized by whey protein isolate (WPI), specifically lactoglobulin complexes with high-methylated (HM) and low-methylated (LM) pectins of various origins. This research examines their stability and adsorption properties in relation to a model drug, with the goal of creating an effective drug delivery system (DDS).

2. **Materials and Methods.**

2.1.Materials

The following reagents and materials were used in the work: HM- and LM-pectin obtained by steam-assisted flash extraction (flesh hydrolysis) method [32], from sunflower heads apple pomace and commercial citrus pectin L-12 CG (CP Kelco, USA). β-Lactoglobulin concentrate (LgC) isolated from whey according to procedure [33] contained 37.35% β-LgA, 52.9% β-LgB, and 9.7% α-LgA. Protein sericin extracted with hot water [34] from wasted cocoons of the silkworm (*Bombyx mori*). The average molecular weight (Mw) of sericin found by the HPLC size exclusion chromatography method [35] was 24.0 KD. Polyphenolic compounds (PPhC) of propolis from a bee farm in the Yavan district and pomegranate peel grown in the Republic of Tajikistan, obtained by extraction with a 70% ethanol solution, were used as BAC [36].

The non-steroidal anti-inflammatory drug- piroxicam (PX) (4-hydroxy-2-methyl-1,1-dioxo-N-(2-pyridinyl)-2H-1,2-benzothiazine-3-carboxamide) was used as a model drug.

2.2.Preparation of microcapsules

A two-stage procedure to obtain double-layered emulsion microcapsules was applied as described in reference [18]. Briefly as follows: primary emulsion - homogenization of the initial oil in water mixture with B-LgC or sericin solutions on a high-speed homogenizer IKA T-25 (Ultra Turrax, IKA-WERKE GMBH & CO.KG, Germany) followed by adding a pectin solution to form second layers.

2.3.Characterization of microcapsules

The particle size and the number of microcapsules in an emulsion system with lgC and pectin were measured on a digital biological microscope Motic type 102 M (Motic Instrument INC, Canada); the number of particles and their sizes were determined on a microscope using the computer program Motic Image Advanced 3.2. The particle diameter of microcapsules in the dilute emulsion was measured by a light microscope instrument and calculated with Motic Image Advanced 3.2 software.

The stability of the microcapsules in emulsion systems with sericin/pectin was assessed microscopically using an OLYMPUS BX53 microscope (OLYMPUS U-TR30-2, Japan). The

number of macroparticles and their sizes were determined by the OLYMPUS cell Sens Standard computer program.

The particle size was the volume-weight average diameter d_{43}, which was calculated using the formula (1):

$$d^{43} = \sum nidi^4/\sum nidi^3 \qquad (1)$$

where n_i is the number of emulsion particles with a diameter d_i, the particle size was also reported as the mean particle diameter d_{43}. The particle numbers were calculated by using the Excel pregame from the particle size numbers.

The actual drug loading was determined by calculating the total amount of drug applied to the emulsion system minuses of the washed-out amount by subsequent UV drug detection. All measurements were made on at least two freshly prepared samples and results were reported as means and standard deviations.

2.4.Determination of antioxidant activity

The total antioxidant activities of PPhs and emulsion were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay described in [37] with minor modification. The PPhs or emulsion containing that were dissolved in ethanol (25 μg mL^{-1}) and mixed with 0.2 mM DPPH radical solution. Ascorbic acid (AA) was used as a reference compound. All measurements were done in triplicate. The absorbance was measured at 517 nm, and DPPH scavenging activity was determined using Equation (2):

$$\text{DPPH activity} = (A_b + A_s) - A_m)/A_b \times 100 \qquad (2),$$

where, A_b is the absorbance of 0.1 mM DPPH radical solution at λ = 517 nm, A_s is the absorbance of 0.1 mM PPhS solution at λ = 517 nm, and A_m is the absorbance of 0.1 mM solution mixture of tested extracts and DPPH radical at 517 nm.

3. Results and Discussion

3.1.Preparation of emulsion microparticles in the system of LgC and HM- and LM-pectin for drug delivery purposes

The pectin samples used to prepare the microcapsules were obtained by flash extraction and differed in AGA content, DE, molar mass, molecular mass distribution, and hydrodynamic dimensions. Table 1 presents the main characteristics of the studied pectin samples.

Table. 1.

The pectin's main characteristics (include galacturonic acid (AGA), degree of esterification (DE), molecular weight, and hydrodynamics parameters).

Pectin samples	AGA, %	DE, %	MG, %	M_w	M_w/M_n	h_w	R_w
HMOP 120-10	69,00	78,00	3,70	96,40	14,20	78,00	8,40
HMAP 120-7	68,00	52,00	4,70	160,00	3,80	150,60	13,30
LMSP 120-5	82,00	35,50	4,50	72,60	3,06	61,30	8,00
LM-12CG (CP Kelco)	69,00	31,0	2,10	136,00	2,85	180,00	14,00

A wide range of microcapsules in a whey LgC system with both HM- pectin, obtained from orange and apple, LM- pectin from sunflower head residue, and commercial citrus pec-

tin from CP Kelco Company (USA) with different biopolymers ratios were produced. Microcapsules were characterized by stable emulsion volume, pH, drug loading efficiency, volume average mean particle diameter (d_{43}), and number of particles in 1 ml of emulsion.

3.2.Characterization of emulsion microparticles

Microcapsules formed with orange HM - pectin (Table 2.) have stabile emulsion: the emulsion volume increased from 16.6 to 30.0 ml by decreasing LgC/pectin ratio. The maximum PX loading is 64.13% in LgC/pectin molar ratio of 40. More stable microparticles, containing maximal particle number 237224 per ml emulsion and minimum sizes, formed in the system with a biopolymers ratio of 20. Although the further increase in biopolymers ratio produces a more stable emulsion, it decreases piroxicam (PX) loading and particle number and increases the particle size.

Table 2.

The characterizations of microcapsules produced from orange HM-pectin (HMOP)

LgC/Pectin mol/mol	V, ml emulsion	pH emulsion	PX loading, %	D_{43}, μm	N, particle number
40.0	16.6	4.08	64.13	13.0	147121
20.0	18.6	4.18	62.74	7.00	237224
12.0	30.0	4.99	47.21	15.5	42034

The microcapsules tailored from LM-citrus pectin (LM-12CG) show the same features of stability depending on the biopolymer's ratio but less stability in the volume of emulsion (14.8-21.0 ml) in the range of biopolymers ratio (54.0 -12.0 mol) consequently (Tabl. 3).

Table 3.

The characterizations of microcapsules produced from citrus LM-pectin (LM-12CG)

LgC/Pectin mol/mol	V, ml emulsion	pH emulsion	PX loading, %	D_{43}, μm	N, particle number
54.0	14.8	3.67	62.1	5.28	12113818
20.0	18.8	3.96	63.4	9.59	8772075
12.0	21.0	4.10	53.1	8.02	1069523

In this emulsion system with pectin, the variation in the number of microparticles and their average diameter, depending on the biopolymer ratio, follows a distinct pattern. The number of particles increases to a maximum, at which point 54 particles with a minimum diameter of 5.28 μm are formed. A further increase in pectin within the secondary layers of microparticles results in a stable emulsion with smaller particle sizes; however, the PX loading capacity decreases.

In the case of microcapsules produced in systems with LgC and apple pectin (HMAP), higher stability was observed with a decrease in the polymer molar ratio from 40 to 15. A stable emulsion at a biopolymer molar ratio of 15 exhibited a very high number of microparticles (1,769,651) with small particle sizes (4.5 μm) while capturing the maximum amount of drug (66.04%). As shown in Table 3, a further decrease in the LgC/pectin ratio adversely affects all studied parameters.

Table 3.

The characterizations of microcapsules produced from apple HM-pectin (HMAP)

Lg/Pectin	V, ml	pH	PX	D_{43},	N, particle

mol/mol	emulsion	emulsion	loading, %	µm	number/ml
50	18.0	3.95	57.06	9.0	147121
33	19.8	4.78	57.03	8.5	165700
25	19.2	3.66	53.92	8.0	277000
15	23.0	4.02	66.04	4.5	1769651
10	19.8	4.13	63.43	8.5	299000
6	18.6	4.78	40.76	14.0	89700

For the system with sunflower pectin, such a relationship between the ratio of biopolymers and the characteristics of the obtained microcapsules was observed (Table 4). The decrease in protein fraction in the produced microparticles from the biopolymer ratio of 40 to 16 mole leads to an increase of all parameters in the emulsion system. The optimal molar ratio of here was 16, where the optimal parameters are observed.

Table 4.

The characterizations of microcapsules produced from sunflower LM-pectin (LMSP)

Lg/Pectin mol/mol	V, ml emulsion	pH emulsion	PX loading, %	D_{43}, µm	N, particle number
40	15.8	4.00	38.05	13.8	165000
20	16.6	4.37	43.73	10.4	207000
16	14.6	4.60	61.48	7.8	389000
12	18.0	4.50	65.00	8.2	363000

Unlike the emulsion system with LM-citrus pectin, the number of particles in the LgC/LMSP emulsion system at an optimal molar ratio of 16 reached 389,000, which is almost three times less than that of the first system. The formation of relatively large particles with LM-citrus pectin is possibly associated with the pectin's structure (branching of the macromolecule) and charge density.

The change in the biopolymer ratio in the presence of HMOP and LMSP does not significantly affect the pH of the formed emulsion ($P > 0.05$). However, in the system with LM-12CG and HMAP, the pH of the emulsion slightly increases with the rise in pectin fraction.

Previous studies [5, 17, 18] have shown that unstable emulsion microcapsules obtained in the LgC/LM-citrus pectin system with high pectin content. Oil droplets surrounded by Lg aggregate upon adding low-methylated pectin with a high chain charge. With an increase in the pectin fraction, particles with a smaller diameter are observed. An increase in the protein fraction beyond this point (LgC/pectin 12) likely leads to charge compensation of the pectin chain and a decrease in the total charge on the secondary layer, which disrupts emulsion stability.

Consistent with the results of this study, optimal conditions have been identified for obtaining stable microcapsules in the oil/water emulsion system at different ratios of both LgC/HM- and LgC/LM-pectins. These conditions yield a minimum particle size and a maximum number of particles per unit volume while effectively capturing the drug substance. It has been demonstrated that the formation of a stable emulsion with a high degree of drug encapsulation depends on the type of pectin, charge density, molecular weight, environmental conditions (pH and ionic strength), biopolymer ratio, and aggregation capacity. Among the studied pectins, LM-pectin is the most capable of forming stable emulsions while effectively encapsulating the drug substance over a wide range of biopolymer ratios.

The developed systems for delivering drugs in the form of emulsion microparticles have demonstrated the ability to effectively capture them, which is important when creating delivery systems for injectable and aerosol drugs. The mechanism of action of such a system is based on the fact that emulsion microparticles carrying the drug, stabilized by a protein-pectin complex, are able to protect the drug from the effects of an acidic environment and enzymes of the upper gastrointestinal tract. The multilayer LgC-pectin complex, located on the surface of oil microparticles, will prevent swelling of the pectin layer and rapid drug release. It is assumed that such a biopolymer layer decomposes in the large intestine, from where the drug enters the bloodstream. However, it should be taken into account that the rate of drug release will also depend on many factors, including the type of pectin and pectin-protein complex, the packing density of the polymer layer, and the structure of the biopolymers themselves, as shown in our previous studies. [5, 17, 18, 19, 38-40].

3.3.Preparation and Characterization of emulsion microparticles in the system of Silk Sericin and HM-apple pectin for BAC delivery purpose

Microcapsules based on sericin protein and pectin for encapsulating BAC were obtained using the technology of multilayer emulsion. Table 5 presents the characteristics of emulsion microcapsules obtained by the new technology based on the emulsion, stabilized by complexation of HM-apple pectin (HMAP) with silk sericin protein (SSP), containing the BAC-polyphenol compounds of propolis.

Microcapsules were evaluated for volume stability over time. The total volumes of the resulting emulsions at all SSP/HMAP ratios were initially 20 ml, decreased the next day to stabile emulsion, and after a few days, a compact, creamy layer was formed (3.2-4.0 ml).

Table 5.

The characterizations of microcapsules produced from LM-apple pectin and silk sericin [30]

SSP/HMAP mol/mol	Volume of emulsion, mL	pH emulsion	PPhC loading, %	D_{43}, µm	N, particle number
13.7	15.7	3.60	42.81	5.95	830000
22.5	18.4	3.64	47.43	5.67	917000
45.5	15.4	3.65	46.29	6.79	389000

The most important characteristics of many dispersed systems (especially oil-in-water emulsion systems) are the size and shape of the elements of the dispersed phase since most of the other properties of such systems depend on these parameters.

The results of calculating the average diameters of microcapsules and the volume fraction of particles (V, %) for different SSP/NMP ratios are given in Table 5 and figure 1.

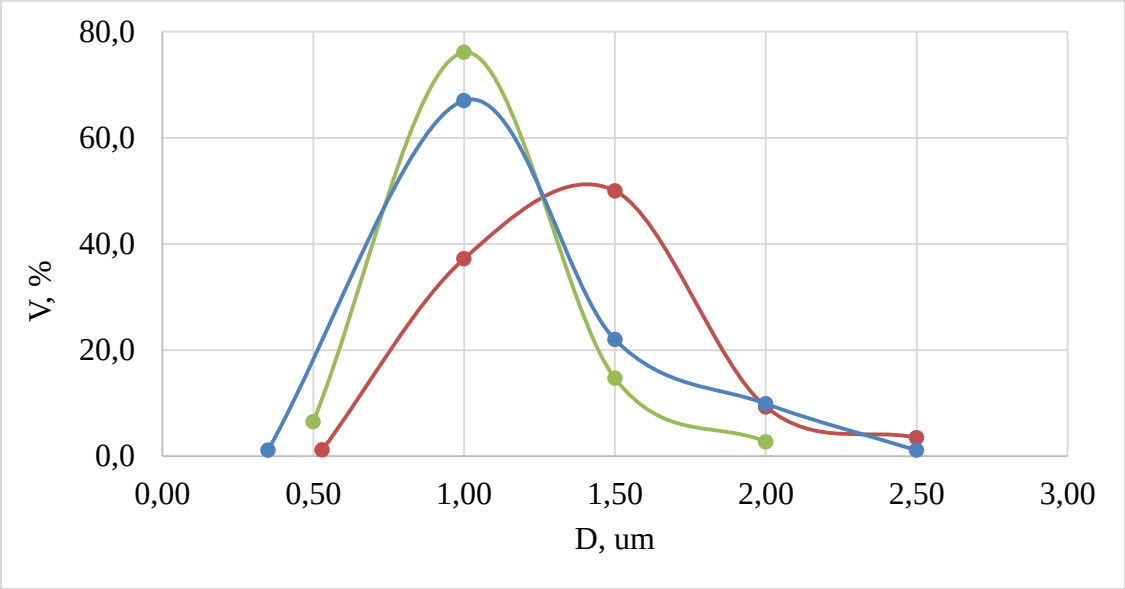

Figure 1. Distribution curves of layer microparticles in the emulsion system of sericin and NM-pectin with weight ratios of SSP/HMAP (mol: mol) – 13.7 (---orange line), 22.5 (---black line) and 44.5 (---blue line)

The results obtained in this study show that the volumes of emulsions at different ratios remain stable for several days. With an increase in the proportion of sericin protein in the SSP/HMAP system, the stability of the emulsion increases and reaches a maximum value at a protein/pectin molar ratio of 13.7 and then decreases again. Also, the adsorption of PPhC passes through a maximum, reaching a value of 47.43% and decreasing to 37.99% with an increase in the SSP/HMAP molar ratio.

As can be seen from the profile of the distribution curves of the particles of the cream layer (Fig. 2) at the ratio of SSP/HMAP 10:1, which corresponds to the molar ratio of protein and pectin of 45.5, the maximum of the curves is distributed within the range of 1.0 -1.25 μm. It makes up more than 40% of the total volume of particles. At the same time, a decrease in the molar ratio of SSP/HMAP leads to the formation of nano- and microparticles of relatively large size, making up more than 50% of the total volume of particles with a maximum of particles with a size of 1.5 μm. The distribution of the particles of the emulsion's lower (transparent) layer for molar ratios of SSP /HMAP 45.5 and 22.5 covers nanoparticles with sizes of 350 nm to 1.5 μm, making up 70% of the total volume of the emulsion. In this case, a decrease in the proportion of protein on the surface of the particles leads to an increase in particle size and to flocculation of the particles, which leads to an increase in the phase of the silicon layer.

As a result of the studies have found optimal conditions for obtaining stable microcapsules in an oil/water emulsion system at different SSP /HMAP ratios with a minimum size and maximum number of particles per unit volume, effectively capturing PPhC. Silk sericin can form stable emulsions with HM-apple pectin in a wide range of SSP /HMAP ratios of 13-20 mole.

3.4. Estimation of antioxidant activity of the microcapsules

We evaluated the antioxidant activity of emulsion microcapsules with PPhC stabilized by a complex of sericin and pectin, as described above. The antioxidant activity of the polyphenol extract before and after encapsulation was assessed using DPPH analysis. The data are presented in Table 5.

Table 6.

Antioxidant activity (AO) of emulsion microcapsules with propolis polyphenolic compounds

PPhC and SSP **/HMAP mol/mol**	**Dilution of samples**	**AO, %**
PPhC extract from Propolis, 5 mg/mL	1:10	54.32
	1:30	62.03
	1:50	33.71
	1:100	22.84
13.7	Emulsion original	9.01
	1:10	63.66
	1:50	88.32
	1:100	98.23
	1: 500	55.46
22.5	Emulsion original	9.01
	1:10	64.08
	1:50	85.44
	1:100	97.56
	1: 500	60.32
45.5	Emulsion original	9.01
	1:10	24.07
	1:50	80.45
	1:100	98.26
	1: 500	45.81

In the propolis sample from three regions of Tajikistan, other phenolic compounds such as caffeic, gallic and 3,4-dihydroxybenzoic acids were detected. Phenolic compounds such as homogentistic and gallic acids were similar in all samples [25]. As can be seen from the data in Table 6, PPhC from propolis extract showed an ability to scavenge a radical of DPPH (AO activity) at about 54.32%; with a dilution of solutions, the AO slightly increased and then decreased at 50- and 100-fold dilution.

The AO of emulsion microcapsules without PPhC showed low values of DPPH radical inhibition of 9.01%, and at 100-fold dilution, this value reached 19.88%. We found that encapsulation of PPhC as an emulsion in an oil-in-water system exhibits insignificant ($P<0.05$) antioxidant activity in the initial state.

While at dilution, encapsulated PPhC in emulsion microcapsules stabilized by sericin protein and apple pectin contributed to high values of DPPH radical inhibition. After dilution of the emulsion with water, the percentage of free radical trapping increases significantly ($P<0.05$) due to the intensification of PPhC diffusion from the microcapsules. This trend of activity growth is almost the same for all emulsions with different SSP /HMAP ratios. The AO of microcapsules increases from 63±1.7% at 10-fold dilution to 97±1.7% activity as dilution progresses, even at 100-fold dilution of the emulsion.

In accordance with the results of the studies, we have established that propolis extracts contain the highest amount of PPhC, including phenolic acid and flavonoids. PPhC encapsulated in emulsion microcapsules, stabilized by sericin protein and apple pectin, showed high antioxidant activity at a minimum concentration, confirming the preservation of their therapeutic properties. It was found that obtaining an emulsion with a high degree of encapsulation of polyphenolic compounds at 100-fold dilution demonstrated high antioxidant activity.

4. Conclusion

The process of microcapsule formation based on the complexation of LM- and HM-pectin's with different structures, isolated from apples, sunflower, and citrus fruits with whey and silk proteins on the surface of emulsion particles in the oil-in-water (o/w) system was created. The obtained microcapsule is capable of effectively capturing drug and BAC. The conditions for obtaining a stable emulsion with high drug or BAS uptake have been determined depending on the pectin structure, molecular weight, environmental conditions (pH and ionic strength), and protein/pectin ratio.

Consistent with the results of the studies, we have found that silk sericin can form stable emulsions with HM-apple pectin in a wide range of SSP /HMAP ratios of 13-20 moles. Also, we have established that propolis extracts contain the highest amount of PPhC, including phenolic acid and flavonoids. PPhC encapsulated in emulsion microcapsules, stabilized by sericin protein and apple pectin, showed high antioxidant activity with a minimum concentration, confirming the preservation of their therapeutic properties. It was found that obtaining an emulsion with a high degree of encapsulation of polyphenolic compounds at 100-fold dilution showed high antioxidant activity.

The obtained results can contribute to the progress in encapsulation technology based on biopolymer matrices, which is necessary for applications in various industries, including pharmaceuticals, food and cosmetics, agriculture, electronics, and molecular diagnostics.

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