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Short communication

Investigation of the effects of stirring rate and surfactant concentration on *in vitro* characterization of *Hypericum perforatum* extract loaded polycaprolactone particles

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Introduction

There is a growing interest in the utilization of medicinal plant extracts for addressing fundamental healthcare needs. However, several challenges are associated with their application, including limited solubility in water and instability, which mainly represent common problems in their practical use. Hypericin is one of the active lipophilic ingredients of *H. perforatum* extract that is sensitive to acidic conditions, light exposure. Insoluble aggregates of hypericin forms in an aqueous environment (Zhang et al., 2020). To overcome these challenges for the extracts, the nano and micro particles have been extensively investigated as drug carrier systems due to their ability to protect sensitive active ingredients from external factors, enhance solubility and biocompatibility of lipophilic active ingredients, and provide improved stability (Teixeira et al., 2022).

The purpose of this study is to establish the optimum preparation method for subsequent investigations focusing on the formulation's stability. Thus, the main aim of this study is to investigate specific preparation parameters that influence the preparation process and characterization of the polycaprolactone particles loaded with *H. perforatum* extract.

Materials and methods

Standard Hypericin (Cayman, Germany), polycaprolactone (PCL) (Mw:80,000 Da) (Aldrich, UK), dichloromethane (DCM) (Lab-Scan, Ireland), Polyvinyl alcohol (PVA) (Mowiol 4-88 Mw: 31,000 Da) (Aldrich, Germany) were used. Voucher specimens of *H. perforatum* (AEF 30944) were stored in the Herbarium of the Faculty of Pharmacy of Ankara University.

Preparation of extracts: 20 g of dried and ground plant samples were extracted with 200 mL of 96% ethanol in a Soxhlet apparatus, evaporated under vacuum using a Rotavapor (Buchi RII, Switzerland) at a temperature not exceeding 40°C, and lyophilized for 24 hours (TRST 4/4 Teknosem, Turkey).

Preparation of the PCL particles were performed by nanoprecipitation method. The organic phase consisted of PCL and extract in ethanol-DCM (1:1 v/v) was injected into the aqueous phase (containing PVA) and mixed by homogenizer. Particles were prepared using different stirring rates ranging from 10.000 rpm to 20.000 rpm (F1-F5). Additionally, different amounts of PVA, were used with concentrations varying from 0.3% to 0.9% (F3a-F3c), while the other parameters were maintained constant.

HPLC method was used for the determination of hypericin from 0.3 w/v% PVA solution and PBS (containing 25% v/v ethanol) (pH 7.4). A validated reversed-phase HPLC (Agilent 1100 series, USA) method adapted from EP10 was employed using an ACE5 C18 column (150x4.6mm, 5 µm) at 590 nm and at 20 ºC.

The amount of encapsulated hypericin (EE%) in the supernatant (0.3% w/v PVA solution) was determined by HPLC (*n=3)* using the following equation:

EE%= [(initial added amount−drug amount in supernatant)/ initial added amount] x100.

In vitro drug release studies were performed by a static method (Ilhan et al., 2022). Particles (equivalent to 4,80 µg hypericin) were placed in 1 mL PBS: Ethanol

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(75:25 v/v) at 37°C and shaken at 75 rpm, 0.8 mL of medium withdrawn to analyse by HPLC method *(n=3)*.

Results and discussion

The encapsulation efficiency of *H. perforatum*-loaded particles was found to be 78.22%±4.16 or higher. To obtain a uniform emulsion by effectively mixing the organic and aqueous phases that form the polymeric particle, rapid stirring providing sufficient energy is required. As the stirring rate was increased in the particles (F1-F5), a corresponding decrease in particle size was observed, reducing from 5077±468 nm to 3617±418 nm (Table 1). However, it should be noted that there was no proportional change observed in the polydispersity index (PDI) values of the particles (n=3) with variations in the stirring rate. A surfactant, such as PVA, plays a crucial role as a stabilizing agent in emulsion formation (Ilhan et al., 2022). The type and concentration of the stabilizer used can affect the formation and stability of the emulsion. At moderate stirring rate, an increase in PVA concentration (from 0.3% (w/v) to 0.9% (w/v)) led to a decrease in particle size from 5278±395 nm to 2947±417 nm. Increasing the surfactant amount resulted in lower PDI values, indicating a narrower particle size distribution (F3c).

Table 1. *In vitro* characterization results of *H. perforatum*-loaded PCL particles (Value ± SD)

Parameter		Size (nm)	PDI	ZP (mV)	EE (%)
Stirring rate					
F1	10000	5077 ± 468	0.34 ± 0.06	-36.8 ± 0.31	78.22 ± 4.16
F2	12500	4587 ± 549	0.50 ± 0.04	-41.2 ± 0.85	85.91 ± 0.61
F3	15000	5278 ± 395	0.51 ± 0.03	-42.53 ± 1.00	86.38 ± 0.76
F4	17500	3724 ± 9,24	0.67 ± 0.22	-38.67 ± 1.53	96.43 ± 1.00
F5	20000	3617 ± 418	0.44 ± 0.10	-41.33 ± 1.88	102.0 ± 3.06
PVA					
F3a	0.3%	5278 ± 395	0.51 ± 0.03	-42.53 ± 1.00	86.38 ± 0.76
F3b	0.6%	3540 ± 282	0.43 ± 0.04	-38.20 ± 3.99	91.78 ± 2.08
F3c	0.9%	2947 ± 417	0.28 ± 0.21	-41.73 ± 0.80	93.42 ± 2.17

High zeta potential (ZP) (±30 mV) is necessary for particles stability. Variations in both stirring rate and PVA concentration were not affected significantly the ZP. The stability of all formulations was maintained (Table 1). The observation of negative ZP in all formulations can be attributed to the presence of negatively charged PCL and PVA used in the formulation. In all particles, stirring rate and surfactant concentration variations increased EE (%) (from 78.22±4.16 to 102.0±3.06 and from 86.38±0.76 to 93.42±2.17, respectively) (Table 1). The *in vitro* release rate of hypericin exhibited an inverse correlation with particle size, wherein smaller particles exhibited higher rates of release (Fig.1). The reduction in particle size due to the increase in both stirring rate and surfactant concentration led to an increased surface area, resulting in an enhanced *in vitro* drug release rate. Significant differences in drug release among the formulations were seen at the 12th hour, and cumulative hypericin release rate was ranged from 68.40±2.11 to 87.20±2.49 at the 48th h.

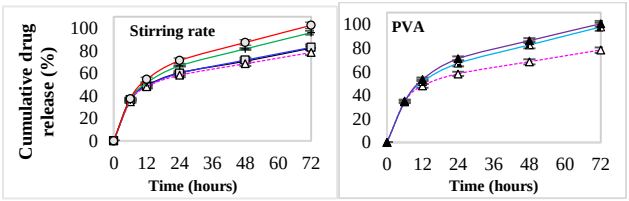

Fig. 1. *In vitro* hypericin release from particles (F1:-◇-, F2: -□-, F3(a): -△-, F4: -|-, F5: -○-, F3b: -▲-, F3c: -▲-)

Conclusion

In conclusion, *H. perforatum* extract was successfully loaded into PCL particles using the nanoprecipitation method. The stirring rate proved to be a significant factor in determining particle size, while PVA concentration influenced both particle size and PDI. Furthermore, variations in stirring rate and surfactant concentration exhibited notable effect on the *in vitro* drug release rate. These findings have provided preliminary data for future studies on hypericin stability in aqueous media.

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