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Original Article

DEVELOPMENT OF CASTICIN-LOADED ETHYL CELLULOSE MICROPARTICLES BY SOLVENT EVAPORATION METHOD WITH SINGLE EMULSION SYSTEM

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ABSTRACT

Objective: Casticin (Vitexicarpin) has shown immunoregulatory, antitumor, cytotoxicity, anti-inflammatory and analgesic properties. Application of the valuable bioactive compounds can be limited by their unpleasant taste, low bioavailability, volatilization of active compounds, sensitivity to the temperature, oxidation and UV light, as well as *in vivo* instability. The problem can be solved by coating the Casticin with a microencapsulation technique. The purpose of this research was to formulate the microcapsules of Casticin with solvent evaporation technique using Ethocel 10 cP.

Methods: The microencapsulation process of Casticin was done by solvent evaporation technique (O/W: oil in water). The formula of Casticin microcapsules were designed into three formulas (Ethocel 10 cP: 10%, 15% and 20%). Microcapsules of Casticin were characterized for particle size, in terms of surface morphology by scanning electron microscope (SEM), encapsulation efficiency and release test.

Results: In this research, the micoparticles containing Casticin has been developed by using ethyl cellulose (Ethocel 10 cP) as the polymer matrix. The results showed that high concentration of polymer (Ethocel 10 cP) used in microencapsulation resulted in better Casticin microcapsules in terms of physical characteristics. Particle size of microcapsules containing Casticin were in the range of 42.51 to 61.47 µm. Encapsulation efficiency (% EE) was categorized as good because the value were ≥ 80% to, which 91.57% to 96.24%. SEM picture of Casticin microcapsules revealed that the surface of microcapsule were a smooth surface and no pores of microcapsule were obtained. When Eudragit E100 used as a polymer, a rough and porous surface of microcapsule were obtained.

Conclusion: It can be concluded that microcapsules of Casticin can be prepared by solvent evaporation method with a single emulsion system (O/W) using Ethocel 10 cP as polymer. Characterization of the microcapsules revealed that ethyl cellulose used on this method is applicable to produce microcapsules which stable in physical properties. A higher polymer concentration led to a more viscous solution, which delayed the polymer precipitation and resulted in a less porous polymer matrix with a slower drug release.

Keywords: Microencapsulation, Solvent evaporation technique, Casticin, Ethocel 10 cP, Release test

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INTRODUCTION

Casticin (Vitexicarpin) has shown immunoregulatory, antitumor, cytotoxicity, anti-inflammatory and analgesic properties [1]. Casticin can act as a novel angiogenesis inhibitor, it exerts good antiangiogenic effects by inhibiting vascular-endothelial-growth-factor-(VEGF-) induced endothelial cell proliferation, migration, and capillary-like tube formation on matrigel in a dose-dependent manner. It can significantly reduce vascular inflammation through inhibition of ROS-NF-κB pathway in vascular endothelial cells. Casticin, a type of flavonoid, could adjust chemical tags on DNA to stave off gastric cancer, a recent study suggests. Casticin (Vitexicarpin) which have been used as medicine several diseases [2, 3]. Casticin, a flavonoid isolated from *Premna serratifolia* Linn leaf.

Application of the valuable bioactive compounds can be limited by their unpleasant taste, low bioavailability, volatilization of active compounds, sensitivity to the temperature, oxidation and UV light, as well as *in vivo* instability [4-10]. One of the potential strategies to overcome these issues is microencapsulation of the bioactive ingredients. Therefore, microencapsulation technology can be used for obtaining bioactive products with desirable characteristics. Microencapsulation techniques of bioactive natural products are widely used in the food, pharmaceutical and cosmetic industries [11-15]. Techniques for the incorporation of bioactive compound within polymer matrices have indicated a good alternative for the improvement of the functionality of medicinal [16-22].

Several methods and techniques are potentially useful for the preparation of microparticles in the field of controlled drug delivery [11-15, 23-31]. The type and the size of the microparticles, the entrapment, release characteristics and stability of drug in microparticles in the formulations are dependent on the method used [32-40]. One of the most common methods of preparing microparticles is the single emulsion technique. Poorly soluble, lipophilic drugs are successfully retained within the microparticles prepared by this method [4-8, 41-46].

The o/w single emulsion solvent evaporation method is the widely used one among various microencapsulation techniques [7, 10, 22]. Water-insoluble drugs are successfully retained within microparticles prepared by this method. However, the method is not efficient for the entrapment of hydrophilic drugs because of rapid dissolution of the compounds into the aqueous continuous phase. Many types of pharmaceuticals and biopharmaceuticals with different physico-chemical properties have been formulated into microparticles by single emulsion method [47-50].

Microencapsulation is a process by which solids, liquids or gases are surrounded with a membrane or matrix [51-53]. Solvent evaporation method has been widely and extensively used to prepare polymeric microparticles containing different drugs and in the development of modified release systems [23-28]. It is a rapid process that does not involve severe heat treatment; therefore, it is a suitable method to preserve biological products, including temperature-sensitive products, without their degradation; it also allows for storage at room temperature [29-34]. It is an instantaneous process where spherical and uniform samples can be obtained, and the process can be easily scaled up [32-37]. The technique of microencapsulation by solvent evaporation is widely applied in pharmaceutical industries to obtain the controlled release of drug. The study included the development of casticin-loaded ethyl

cellulose microparticles by solvent evaporation method with single emulsion system.

MATERIALS AND METHODS

Materials

Casticin (3',5-dihydroxy-3,4',6,7-tetramethoxyflavone, fig. 1 was isolated from the ethanol extract of *P. serratifolia* leaves. Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA), Ethocel 10 cp, gallic acid, ethanol (C_2H_5OH), methanol, dichloromethane, hydrochloric acid, sodium hydroxide, PVA were bought from Merck Chemicals GmbH, Darmstadt, Germany. Naphthylethylenediamine dihydrochloride (PanReac AppliChem, Darmstadt, Germany), Thiobarbituric acid (TBA), casticin (as reference) and Trichloro acetic acid (Sigma-Aldrich, St. Louis, MO, USA). All other reagents used were of analytical grade and were purchased from Sigma-Aldrich (Saint Louis, MO, USA) and PanReac AppliChem (Darmstadt, Germany). UV spectra were recorded in Shimadzu 1601 UV-visible spectrophotometer. The chemicals used were of good quantity and quality standard and do not require further purification.

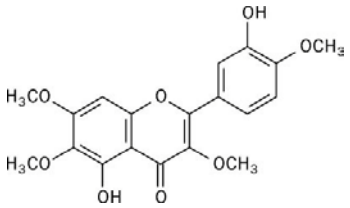


Fig. 1: Chemical structure of casticin

Methods

Preparation of polymeric microparticles

The solvent evaporation method based on the formation of O/W emulsion was used to prepare microparticles. For the O/W method, ethyl cellulose (Ethocel 10 cP (10%,15%, 20%) were dissolved in dichloromethane. 100 mg of Casticin were dissolved within this organic phase. The organic phase was then emulsified into 800 ml aqueous PVA solution (1.5% w/v) containing 0.5 M NaCl and NaOH at pH 12. The emulsion was stirred for 4 h at 500 rpm with a propeller stirrer (Heidolph Elektro GmbH and Co. KG, Kelheim, Germany) to allow microparticle hardening. After 4 h, the microparticles were separated from the external aqueous phase by wet sieving followed by washing with 200 ml deionized water, desiccator-drying for 24 h and storage in a desiccator [10, 14, 21].

Determination of the actual drug loading and encapsulation efficiency

Microparticles (10 mg) were extracted in 1 ml methanol, followed by agitation in a horizontal shaker (IKA HS 501 digital horizontal Shaker, Janke and Kunkel GmbH and Co. KG IKA Labortechnik, Staufen, Germany) for 2 h (n = 3). 0.1 ml of methanol extract was diluted in 10 ml of pH 7.4 phosphate buffer. The polymer was separated from aqueous solution by filtration using filter paper (Whatman®, GE Healthcare UK Limited, Buckinghamshire, UK). Casticin concentration in the obtained aqueous solution was determined by UV-spectrophotometry at wavelengths of 435 nm (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Waldbronn, Germany). The actual drug loading and encapsulation efficiency were calculated as follows [21]:

$$\text{Actual drug loading (\%)} = (M_{act}/M_{ms}) \times 100\% \ldots (1)$$

$$\text{Encapsulation Efficiency (\%)} = (M_{act}/M_{the}) \times 100\% \ldots (2)$$

where M_{act} = actual casticin content in weighed quantity of microparticles, M_{ms} = weighed quantity of microparticles and M_{the} = theoretical casticin content in microparticles.

Particle size analysis

Particle size mean and size distribution of the microparticles were measured by *Dynamic Light scattering* (DLS) (Cilas, 1064 L, France). The appropriate amount of dry microcapsules of each formulation is suspended in deionized water and sonicated for the appropriate time period before measurement. The average diameter of the volume, size distribution and polydispersite of the resulting homogeneous suspension were determined using the DLS technique. The microparticles suspension was dispersed in distilled water and then it was put into the sample chamber of particle size analyzer and measurement of vesicular size was carried out [14, 21].

Scanning electron microscopy

The morphology of microparticles was analysed by scanning electron microscopy (SEM). For surface imaging, the microparticles were fixed on a sample holder with double-sided tape. To investigate the inner structure, the particles were spread on transparent tape and then cut with a razor blade. All samples were coated under argon atmosphere with gold to a thickness of 8 nm in a high-vacuum (SCD 040, Bal-Tec GmbH, Witten, Germany). Samples were then analysed on the scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany) [14, 21].

Stability studies of casticin-loaded ethyl cellulose system

The casticin-loaded ethyl cellulose microparticulate system sterilized by gamma radiation was subjected to stability studies. The stability protocol was designed as per ICH guidelines [22] with certain modification. For the long-term stability of drug products intended for storage in a refrigerator, the conditions of 5±3 °C is suggested in guidelines. We used the same condition for real-time stability analysis of the microparticulate system. For accelerated study, the conditions of 25±2 °C/60% RH±5% RH has been recommended. The irreversible aggregation has been attributed to the residual solvent migration and partial dissolution of the polymer on the superficial layers, leading to coalescence.

In view of this typical behavior, we did not conduct the stability at the accelerated conditions. In light of the above aspects, we found it worthwhile to conduct long-term stability studies for the casticin-loaded ethyl cellulose microspheres. The microsphere samples were packed in amber-colored (5 ml capacity) vials, stoppered with rubber closure, and crimped with an aluminum over-seal. The samples were stored in stability chamber stability chamber TH90G (Thermolab Scientific Equipments Pvt. Ltd., India). After initial analysis of casticin content, the vials were kept at 0 °C and 5±3 °C. The vials were periodically sampled at 1, 2, 3, 6, and 12 mo time period. The stability samples were critically evaluated for physical appearance, particle size analysis, drug content, and real-time *in vitro* dissolution profile. The samples were tested for different parameters. Physical characteristics of the samples were carefully observed for changes in color and clumping/aggregation behavior. Particle size was calculated by optical microscopic method. The possibility of any shriveling tendency in the microparticles was also examined. Individual samples were subjected to drug content. Moisture content in the samples was found by the Karl Fischer method. In the *in vitro* dissolution studies, the static method was employed, as described earlier.

In vitro drug release studies

10 mg microparticles (particle size: <70 µm) were placed in 10 ml pH 7.4 phosphate buffer (USP XXIV) and shaken at 37 °C in a horizontal shaker (GFL 3033, Gesellschaft für Labortechnik GmbH, Burgwedel, Germany) at 75 rpm. At predetermined time points, 1 ml samples were withdrawn and replaced with 1 ml fresh medium each 7 d, filtered and analyzed [10, 14, 21]. Casticin concentration was detected UV spectrophotometrically at wavelengths of 435 nm, respectively (n = 3) (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Waldbronn, Germany).

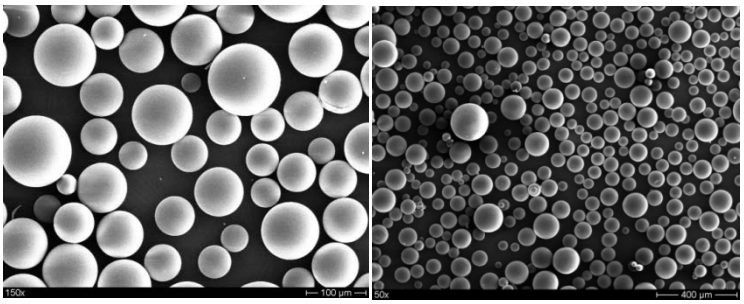
RESULTS AND DISCUSSION

Morphology and particle size/distribution of microparticle

Microencapsulation by solvent evaporation technique is widely used in pharmaceutical industries. It facilitates a controlled release of a drug, which has many clinical benefits. Water insoluble polymers are used as encapsulation matrix using this technique [23, 24, 34]. For

insoluble or poorly water-soluble drugs such as Casticin, an O/W method is suitable used [34].

Scanning electron microscopy was used to examine the microparticles' surface morphology (SEM). Surface analysis of casticin-loaded microparticle generated by the O/W revealed that the microparticles were spherical and not aggregated (fig. 2), with diameters ranging from 42.51 to 61.47 µm. Microparticles created using 400 rpm, produced microparticles with a smooth surface (fig. 2a and 2b).



a **b**

Fig. 2: SEM pictures of casticin-loaded ethyl cellulose microparticle at (a) 1000X magnification and (b) 500X magnification)

SEM photomicrograph of ethyl cellulose (EC) microparticles which were prepared by O/W method, are shown in fig. 2. The surface analysis of microparticles prepared by the O/W method revealed that ethyl cellulose (EC) microparticles were spherical with smooth surfaces, no pores and no aggregation. The particle size mean of microparticles which was prepared using high polymer concentration were larger than those prepared by low polymer concentration. This is caused by rapid solidification process occurring at the surface of embryonic microparticle droplets which resist in extensive shrinkage of embryonic microparticles droplets.

The morphology and smooth of the microparticles were significantly impacted by the preparation procedures. This process involves oil-in-water (O/W) emulsification. The O/W emulsion system consists of an organic phase comprised of a volatile solvent with dissolved polymer and the drug to be encapsulated emulsified in an aqueous phase containing a dissolved surfactant. For insoluble or poorly water-soluble drugs, the oil-in-water (O/W) method is frequently used. This method is the simplest and the other methods derive from this one. It consists of four major steps: (1) dissolution of the hydrophobic drug in an organic solvent containing the polymer; (2) emulsification of this organic phase, called dispersed phase, in an aqueous phase called continuous phase; (3) extraction of the solvent from the dispersed phase by the continuous phase, accompanied by solvent evaporation, transforming droplets of dispersed phase into solid particles; and (4) recovery and drying of microspheres to eliminate the residual solvent [50-53]. Most systems that use oil-in-water emulsions to prepare microparticles consist of an organic phase comprised of a volatile solvent with dissolved polymer and the drug to be encapsulated, emulsified in an aqueous phase containing dissolved surfactant. A surfactant is included in the aqueous phase to prevent the organic droplets from coalescing once they are formed [35-39].

Entrapment efficiency within microparticle

In microparticles, encapsulation efficiency (EE) ranged from 91.57 percent to 96.24 percent for casticin (table 1). The solubility of the medications in the aqueous continuous phase employed for the encapsulating procedures can explain the variation in the EE of casticin in the microparticles.

Table 1: Formulation, drug entrapments and particle size mean of microparticles (whole size)

Polymer concentration (%)	PSA (µm)	Actual drug loading (%) (±SD)	Encapsulation efficiency (%) (±SD)
10	42.51 (±4.15)	14.62 (±0.25)	91.57 (±3.18)
15	53.09 (±3.62)	14.91 (±0.35)	95.72 (±4.66)
20	61.47 (±5.24)	15.46 (±0.29	96.24 (±4.25)

Data are expressed as mean±SD, n=3

Casticin has low stability, so it is formulated in the form of microcapsules by utilizing a polymer that can protect the an active ingredient. The polymer used is ethocel 10 cP with a concentration variation of 10, 15 and 20%, respectively. PVA in microcapsule preparations is commonly used as a polymer stabilizing agent in the solvent evaporation method. However, the use of the polymer must be able to guarantee the stability of casticin, especially in terms of activity.

Based on observations of microcapsules from Casticin using polymer variations and different concentration variations above, we have obtained yields of each polymer with different concentrations.

Stability studies of casticin-loaded ethyl cellulose system

The physical observations of samples, particle size, drug content and moisture content in the casticin-loaded ethyl cellulose microparticles formulation for initial and stability samples at 0 °C are shown in table 2 and at 5±3 °C condition in table 3. The product retained its spherical geometry and did not show shriveling tendency during the 12-month storage period.

Table 2: Stability studies evaluation of casticin-loaded ethyl cellulose system (0 °C)

Sampling time	Physical appearance	Average particle size (µm), avg±SD	Encapsulation efficiency (%), avg.±SD	Moisture (% w/w)
Initial	Free flowing powder	42.51 (±5.24)	91.57 (±3.18)	0.16
1 mo	Free flowing powder	42.11 (±6.52)	92.24 (±4.61)	0.16
2 mo	Free flowing powder	42.47 (±2.78)	90.75 (±4.12)	0.16
3 mo	Free flowing powder	41.88 (±4.74)	91.53 (±3.92)	0.16
6 mo	Free flowing powder	41.02 (±7.82)	92.51 (±5.03)	0.15
12 mo	Free flowing powder	40.97 (±3.63)	91.48 (±3.85)	0.17

All experiments were conducted in triplicate; Polymer concentration (10%)

Table 3: Stability studies evaluation of casticin-loaded ethyl cellulose system (5±3 °C)

Sampling time	Physical appearance	Average particle size (µm), avg±SD	Encapsulation efficiency (%), avg.±SD	Moisture (% w/w)
Initial	Free flowing powder	41.68 (±2.91)	91.82 (±2.74)	0.16
1 mo	Free flowing powder	44.14 (±4.22)	92.38 (±3.62)	0.15
2 mo	Free flowing powder	43.52 (±4.71)	91.93 (±4.12)	0.15
3 mo	Free flowing powder	41.25 (±5.16)	92.31 (±4.18)	0.16
6 mo	Free flowing powder	42.68 (±3.47)	91.83 (±3.55)	0.16
12 mo	Free flowing powder	41.26 (±3.73)	91.47 (±3.26)	0.17

All experiments were conducted in triplicate; Polymer concentration (10%)

The casticin-loaded ethyl cellulose microsphere system was developed in a view to have a system for long-term treatment. In this study, a microparticulate system consisting of the casticin molecule taken as model drug was formulated by a solvent evaporation technique. Stability studies play a major role in defining the safety aspects of the pharmaceutical product and also are a helpful tool in estimating the shelf life of the formulation. Casticin is stable. The stability indicating that there is no increase in the impurity levels in the formulation even after long-term storage.

Effect of polymer concentration on the release of casticin from microparticle

The polymer concentration plays an important role in the drug release from microparticle systems. A decrease in the drug release from microparticle systems with the increasing polymer concentration was already reported [39, 48, 50, 51]. A higher polymer concentration led to a more viscous solution, which delayed the polymer precipitation and resulted in a less porous polymer matrix with a slower drug release. In microparticle systems, casticin release after 28 d decreased dramatically from 92%, 80% and 62% with an increasing polymer solution concentration of 10%, 15%, 15% and 20% (w/v), respectively (fig. 3). Different release rate were observed for casticin from EC microparticle with different polymer concentration (fig. 3).

In many cases, the drug release rate increases with increasing drug loading [39, 48, 50, 51]. There are two possible explanations for the effect of drug loading. First, the elution of surface-associated drug creates water-filled channels that allow subsequent elution of the drugs located inside the microparticles. By facilitating formation of these channels, high drug loadings lead to high initial bursts and fast release rate [39]. Alternatively, a large drug concentration gradient between the microparticles and the release medium may promote high initial bursts and fast release rate [48].

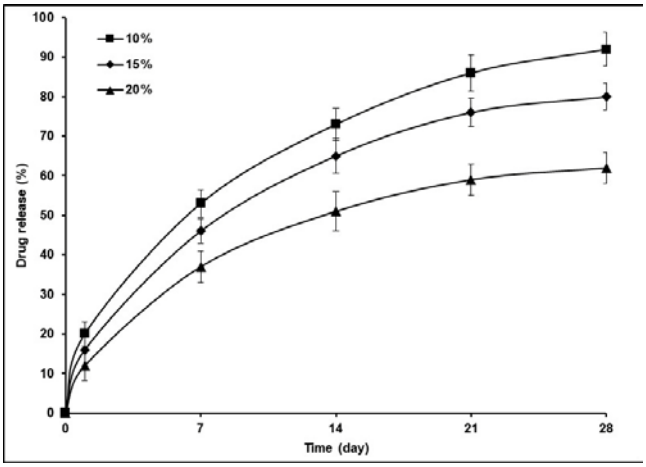


Fig. 3: Effects of polymer concentration on casticin release from ethyl cellulose microparticle (phosphate buffer, pH 7.4, 37 °C, 75 rpm). N=3

The controlled release of drug in pharmaceutical applications can be achieved by the microencapsulation by solvent evaporation technique. The properties of materials and the process engineering aspects strongly influence the properties of microspheres and the resultant controlled release rate. In case of the preparation of polymeric microparticles for sustained drug release by solvent evaporation technique, the solidification rate is a decisive factor for their release behaviour. A very slow hardening of the emulsion droplets leads to the diffusion of the drug substance out of the droplets and encapsulation efficiency becomes low. Solidification rate of polymeric microparticles during solvent evaporation process was influenced solubility of polymers in organic solvents and solubility organic solvent in water, which in turn affects microparticle properties such as particle size, drug incorporation, matrix porosity, solvent residues and initial burst [30-35]. Dichloromethane is the most common solvent for encapsulation using solvent evaporation technique because of its high volatility, low boiling point and high immiscibility with water [23, 24].

Microencapsulation techniques with film polymers can use several kinds of polymers, including Ethocel 10 cP. Ethyl cellulose (EC) is a partly O-ethylated cellulose ether derivative. It is available in a variety of grades, which differ in viscosity, is usually hydrophobic in nature and widely used in the biomedical and pharmaceutical industries. Ethyl cellulose is usually distinguished by viscosity, molecular weight and is referred to as "Ethyl Cellulose Polymer Premium", with the trade name Ethocel TM. Ethocel TM types are ethocel 4, 7, 10, 20, 45 and 100 cP. The one used in this research is ethocel 10 cP because it is most often used in the coating process in the pharmaceutical field.

CONCLUSION

Casticin can be prepared by solvent evaporation method with single emulsion system (O/W) using Ethocel 10 cP as polymer. Characterization of the microcapsules revealed that ethyl cellulose used on this method is applicable to produce microcapsules which stable in physical properties. A higher polymer concentration led to a more viscous solution, which delayed the polymer precipitation and resulted in a less porous polymer matrix with a slower drug release.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

No conflicts of interest is associated with this work.

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