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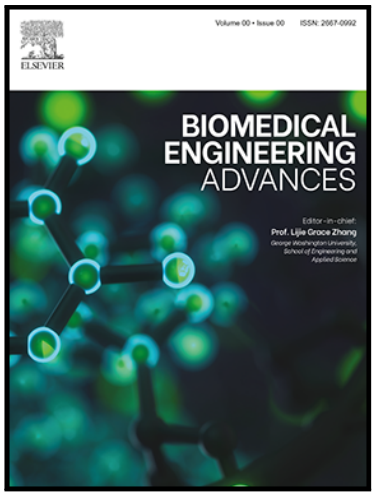

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Sustained Release of Salicylic Acid from Ethyl Cellulose Microspheres Fabricated Using Quasi-Emulsion Solvent Diffusion Method

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ABSTRACT

Delivery of drugs using micro-sized particles is an efficient tool. Using ethyl cellulose (EC) as a polymer and the quasi-emulsion solvent diffusion (QESD) method paves the way for the utilization of a cost-effective polymer and an efficient fabrication method. Poly(vinyl alcohol) in water was used as an external phase while EC and drug dissolved in dichloromethane as an internal phase. Salicylic acid (SA) is known to inhibit prostaglandins and have an anti-inflammatory effect. Furthermore, it inhibits the formation of bacterial biofilm on surfaces. Microparticles of several variations were successfully fabricated using the QESD method in an efficient time frame. These microparticles ranged in size from 5-40 μm for these formulations. Characterization results substantiated the selection of materials used for fabrication. Cytotoxicity showed that these fabrications were biocompatible and did not significantly inhibit cell proliferation. *In vitro* drug release studies showed that the fabricated EC microparticles were able to sustainably release the drugs contained within them over a 96-hour time period, with most of the drug being released after 48 hours. This release never exceeded 63% of the total drug content. PEG addition in the fabrication process of formulation ECPSA eliminated burst release, resulting in a sustained release pattern over 48 hours, reaching saturation by day 5. While entrapment was dependent on the polymer content, increasing drug content did not significantly affect drug release. Behavioral studies evaluated the microspheres, revealing different kinetic models, including Higuchi in ECSA2 and Korsmeyer-Peppas in ECSA3, ECSA5, ECSA6, and ECPSA, with varying diffusion mechanisms. Future fabrications can be made to increase the porousness of the spheres to increase drug release from the microparticles. Then, drug release studies can be conducted over longer periods of time, and with nearly infrared light (NIR) to observe the changes in drug release between the fabrications.

1. Introduction

Dosage control, specifically for controlled release is very crucial for prolonged therapeutic effect. Microspheres being used as carriers are extensively researched for medicinal applications. There is a prevalent use of biocompatible materials to fabricate such microspheres. Microsponges are non-collapsible, porous, polymeric microspheres with a particle size range of 1 to 300 μm that have the capacity to entrap a variety of active substances and release them gradually.

Ethyl cellulose (EC) microspheres were previously used for the release of anti-HIV drug zidovudine [1], pesticide norfluazon [2], stavudine [3], a highly water-soluble drug-fenoterol hydrobromide [4], acyclovir [5], naproxen sodium [6], ketoconazole [7], diclofenac sodium [8], theophylline [9, 10], etc. EC microspheres were used in conjunction with scaffolds and cross-linked with other polymers to make them more efficient at bio-degradation. EC/chitosan microspheres and EC/chitosan hybrids cross-linked with the aid of genepin, a natural bio-crosslinker, have been employed for the targeted delivery of domperidone [11] and as anti-tuberculosis agents [12], respectively. Functional inorganic titanium nanoparticles were incorporated into EC microspheres for use in enhancing light-scattering [13]. While EC microspheres can be used stand-alone as a delivery carrier, they can be embodied into films [14, 15] or scaffolds [16] to further control the release of the drugs that are contained within them for applications such as wound healing and bone regeneration. In controlled-release drug delivery systems, EC microspheres are used to encapsulate drugs, and their properties, such as particle size and surface charge, are adjusted to regulate the rate of release. It has a low rate of water retention and limited water solubility, both of which contribute to a controlled release of drugs in the body [17]. Additionally, it has a better resistance against acidic and alkaline conditions [11]. EC polymer is known to be biocompatible *in vivo* with relatively low immune reaction, substantiated by the superiority in attachment, viability, and proliferation of human bone mesenchymal stem cells [18, 19] on a titanium alloy (Ti6Al4V) substrate. Due to their biocompatibility, EC has been used in the fabrication of films [20, 21], microspheres [22], microcapsules [23]. In general, EC microspheres are a helpful material because of their capability to boost the performance of active ingredients, their adaptability, and the control that they have over the release of drugs and other active compo-

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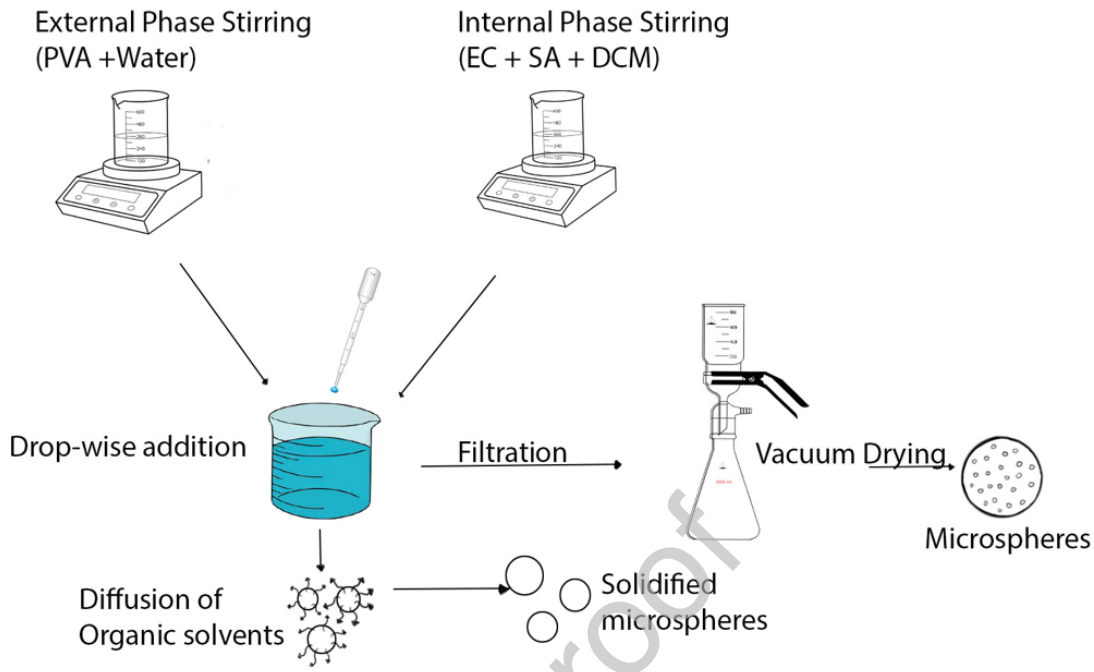


Method.pdf

Figure 1: Schematic of quasi-emulsion method used to fabricate EC microspheres with SA. EC: Ethyl Cellulose, PVA: poly(vinyl alcohol), SA: Salicylic Acid, DCM: Dichloromethane.

nents.

Salicylic acid (SA) is a naturally occurring phytochemical that has beneficial biological properties such as inhibition of prostaglandin synthesis, anti-oxidative properties, anti-inflammatory effects, and nuclear factor kappa B inhibition. [24]. A study done by Ruffin *et al*. showed that aspirin (acetylsalicylic acid) used for colo-rectal cancer for inhibition of prostaglandins showed a six-fold lower half-life when compared to its metabolite SA [25] and can be obtained via a plant-based diet [26]. The elimination half-life of salicylate in adults ranges from 2.4 to 19 hours [27]. In a study on New Zealand sheep, the elimination half-life of sodium salicylate was reported to be 30 minutes after intravenous administration [28]. Salicylic acid is classified under the Biopharmaceutics Classification System (BCS) as Class I [29]. BCS Class I drugs are distinguished by their highly desirable characteristics for oral absorption, which include excellent solubility and permeability [30]. This classification indicates that salicylic acid has a high potential for effective and efficient absorption into the bloodstream following oral administration [31]. The combination of good solubility and permeability enhances its bioavailability, allowing it to readily enter the systemic circulation and exert its intended therapeutic effects. It is important to note that the pharmacokinetic parameters of salicylic acid may vary depending on the dose, route of administration, and species being studied. The effect of SA on skin has been positive and has been shown to have coherent satisfactory cutaneous benefits [32, 33, 34]. While the research on defense associated mechanism of SA on plants [35, 36] and animals [37], it is highly limited in humans and a possibility of overlap is expected with it being a naturally available product [38]. SA has been used for several skin diseases because of its keratolytic, fungicidal, and bacterio-static properties [39, 40].

The originality of this research lies in the use of EC microspheres to release SA sustainably. The main aim is to develop the use of EC microspheres containing SA for modulating a systematic release pattern. EC microspheres are formulated using the quasi-emulsion method employing dichloromethane (DCM) as a solvent for the internal phase and poly(vinyl alcohol) (PVA) for the external phase. EC microsphere fabrication procedure is relatively cheap and easy, making it an ideal candidate for the sustained release of drugs for longer periods. To make the EC microspheres more biodegradable, poly(ethylene glycol) is added to them. Furthermore, the incorporation of Ag NPs diversifies the applications of the formulated microspheres. The microspheres are analyzed in order to determine the production technique and particle qualities that are optimal.

2. Materials and Methods

2.1. Materials

Poly(vinyl alcohol) (PVA) (87-90% hydrolyzed with an avg. molecular weight of 30,000-70,000), dichloromethane (DCM), Ethyl Cellulose (EC) (48.0-49.5% (w/w) ethoxyl ba-

Table 1
Varying concentrations of initial polymer/drug/Ag NPs for fabrication of different formulations. EC, SA, PEG, and Ag NP's content in mg; DCM and PVA content in ml.

	ECSA1	ECSA2	ECSA3	ECSA4	ECSA5	ECSA6	ECSAAg	ECAg	ECPSA
EC	0.4	0.4	0.4	0.1	0.2	0.3	0.4	0.4	0.4
SA	0.2	0.3	0.1	0.2	0.2	0.2	0.2	-	0.2
Ag NPs	-	-	-	-	-	-	0.01	0.01	-
PEG	-	-	-	-	-	-	-	-	0.2
PVA	200	200	200	200	200	200	200	200	200
DCM	40	40	40	40	40	40	40	40	40

sis), and Salicylic Acid (SA) (practical grade ≥ 99.0%) were obtained from Millipore Sigma, USA. Ag NPs were purchased from Sky Nanomaterials, USA. Human embryonic kidney cells (HEK293) and poly(ethylene glycol) (PEG) (MW: 8000) were supplied by the Bioengineering Department, University of Massachusetts, Dartmouth. All chemicals used were of analytical grade without further purification.

2.2. Methods

2.2.1. Fabrication of EC Microspheres

It involves the following steps: A solution of the polymer to be used for the microspheres is mixed with a solvent that is immiscible with the continuous phase (usually water). This creates a droplet-based emulsion. The emulsion is then added to a water-based solution containing a surfactant. The surfactant adsorbs at the droplet-water interface, which helps to stabilize the emulsion. The solvent within the droplets then diffuses out into the continuous phase, causing the droplets to shrink and the polymer to solidify. The microspheres are then collected by centrifugation or filtration and can be washed to remove any remaining surfactant or solvent. The EC microspheres were fabricated using a quasi-emulsion method. 1% w/v EC was dissolved in dichloromethane (DCM) and added drop-wise to a spinning solution of 1% w/v poly (vinyl alcohol) (PVA). The microspheres were collected through filtration and dried in a vacuum desiccator at room temperature. To prepare drug-loaded microspheres SA was dissolved along with EC in DCM. Various formulations were developed with different drug and polymer content, while maintaining a constant water phase. The rationale behind using different formulations was to investigate the impact of varying drug and polymer concentrations on the desired properties of the final product. The initial three formulations, namely ECSA1, ECSA2, and ECSA3, involved keeping the polymer content constant while altering the drug content. This approach allowed for evaluating the influence of different drug concentrations on the characteristics of the formulation, such as drug release rate, stability, and overall performance. In the subsequent three formulations, ECSA4, ECSA5, and ECSA6, the focus shifted to modifying the polymer content while maintaining a consistent drug content. By doing so, the specific impact of varying polymer concentrations on critical aspects, such as particle size, encapsulation efficiency, and drug-polymer interactions, could be assessed.

This systematic approach of altering either the drug or polymer content in a controlled manner provides valuable insights into the formulation design, enabling optimization of the final product based on the desired properties and performance requirements.

2.2.2. Production Yield (PY) of the Microspheres

Production yield is an important parameter to judge the efficiency of the method used for the preparation of microspheres. It is the percentage of the total mass of the product obtained from the total mass used which can be calculated from the following formula:

$$PY = \frac{Practical\ Mass\ of\ Microsponges}{Theoretical\ Mass\ (Drug + Polymer)} * 100 \quad (1)$$

2.2.3. Percent Drug Content and Entrapment Efficiency

The quantification of percent drug content and encapsulation efficiency for the prepared microspheres involved weighing samples of the microspheres and dissolving them in a 0.1 N HCl buffer. The solution was subjected to sonication at 30°C for 4 hours and then filtered. The resulting solution was analyzed at a wavelength of 305 nm. The percent drug content and entrapment efficiency were determined using the following equations:

$$Drug\ Content\ (\%) = \frac{M_i}{M_{mp}} \times 100 \quad (2)$$

$$Entrapment\ Efficiency\ (\%) = \frac{M_i}{M_t} \times 100 \quad (3)$$

where M_i is the amount of drug present in the fabricated microparticles, M_{mp} is the weight of the fabricated microparticles, and M_t is the theoretical amount of drug in the microparticles.

2.2.4. Drug Release Assay

To test for drug release from the microspheres, 10 mg of fabricated EC microspheres containing SA were placed into a 2 ml solution of phosphate-buffered saline (PBS) with a pH of 7.4 inside a Cytivia dialysis kit with a cut-off of 8 kDa. The Cytivia kit containing the solution was vortexed for dispersion of the microspheres and placed in a water bath

containing 18 ml of the same PBS at 37°C. 1 ml of the sample was taken out at specific time intervals. To maintain the sink condition, 1 ml of PBS was added to the solution. To ensure homogeneity, a consistent stirring speed of 60 rpm was employed. The absorbance of the obtained samples was assessed using a SpectraMax Plate Reader, specifically at a wavelength of 305 nm. This process was repeated three times and subsequently analyzed.

2.2.5. Microspheres Biocompatibility Using alamarBlue

To test the biocompatibility of the fabricated microspheres alamarBlue, a cell viability assay reagent was used. 0.0251 g of Resazurin sodium salt was weighed in a 120 ml beaker and 100 ml of deionized water was added to it. The container was wrapped in aluminum foil and filtered using a syringe filter in the cell culture hood with lights turned off. The stock solution was then wrapped in a foil and stored in the fridge in order to prevent it from getting exposed to light. Microspheres with different formulations were added to HEK 293 at a concentration of 0.1%. 24 hours after cell seeding, a working solution containing a pre-determined volume (for 18 ml of working solution add 3.6 ml of prepared solution and 14.4 ml of media) of Resazurin solution was prepared. The media was aspirated from the 6-well plates, and subsequently, 2 ml of the working solution was added to each well. Following this step, the well plate containing the samples was carefully covered with aluminum foil and placed in an incubator set at 37°C with 5% CO_2 for a duration of 2 hours. After the incubation period, 200 μl of the reduced working solution were transferred from each well to a separate 96-well plate.The fluorescence readings were obtained using an excitation wavelength of 540 nm and an emission wavelength of 590 nm. This procedure was subsequently repeated at time intervals of 48, 72, and 90 hours.

2.2.6. Characterization of Microspheres

A spectrometer (Cary 630 FTIR, Agilent, Santa Clara, CA, USA) was used to analyze the infrared spectrum of the samples. The sample was placed onto the apparatus and the Attenuated Total Reflectance (ATR) objective was made to touch the sample. The absorbance readings generated from the surface of the sample were then recorded.

To analyze the shape and size of the fabricated microspheres a HITACHI 3700n-VP Scanning Electron Microscopy(SEM) was used to take the images. A low voltage of 2 kV and a magnification of x300 was used to take all the images. Dimensions of the microspheres fabricated using different concentrations of drug/polymer and Ag NPs as shown in Table 1 were observed using the SEM.

The particle size distribution (PSD) was calculated using ImageJ software (platform-independent software developed at the National Institute of Health (NIH), USA), wherein a sample size of 200 microspheres was taken manually and analyzed.

2.2.7. Micrometrics of Fabricated Microspheres

Micrometric analysis of different formulations of fabricated microspheres was done. Different parameters such as tapped density (TD), bulk density (BD), HausnerâĂŹs ratio (HR), CarrâĂŹs index (CI), and angle of repose were measured. These parameters are defined as:

$$TD = \frac{Mass}{V_t} \tag{4}$$

$$BD = \frac{Mass}{V_b} \tag{5}$$

$$HR = \frac{TD}{BD} \tag{6}$$

$$CI = \frac{TD - BD}{BD} * 100\% \tag{7}$$

where V_t is the volume occupied by the powder after tapped 500 times and V_b is the volume occupied by the powder before tapping.

2.2.8. Adding Poly(Ethylene Glycol) and Silver Nanoparticles

The same process as described above was used to fabricate microspheres containing PEG. PEG was added to the internal phase of the emulsion process. Since PEG dissolves in aqueous solutions, the timing was shortened and the DCM was evaporated in a water bath at 37°C. Ag NPs were added to the internal phase when fabricating the EC/SA and EC-only microspheres.

3. Results and Discussion

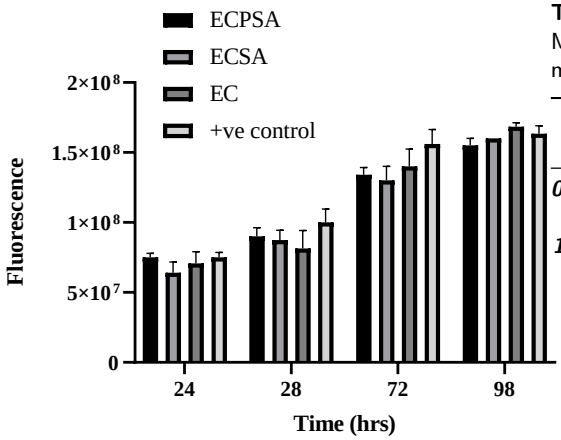
3.1. Microspheres Cytotoxicity Analysis Using alamarBlue

The results from the cell cytotoxicity assay are shown below in Figure 2.

There is no significant difference in cell growth between the different formulations and the positive control (only seeded cells). The growth is very similar at 24, 48, 72, and 98 hours. The alamarBlue assay highlights the biocompatibility of the fabricated particles. In a 5-day period cytotoxicity test, the cells seemed to thrive with the microspheres in the media. In order to ensure that the microspheres are not discarded when changing the cell media, different culture plates were used at different times in the same conditions.

3.2. Production Yield of Microspheres

The production yield of the microspheres varies with changes in the concentration of polymer and drug. In Figure 3 A, we can see that the highest production yield was for ECSA1 where the polymer-to-drug ratio is 2:1. Fabricating microspheres without the use of SA further increased the production yield as seen in Formulation ECAg. SA is water soluble



Blue.pdf

Figure 2: Cell viability assay using alamarBlue for different formulations of fabricated microspheres.

and stirring for a long period of time, subsequently led to the reduction of the production yield. Also, the filter paper used had a cut-off of 5 μm and any particle size below that was filtered out which could have further led to a reduction in the overall weight of the microspheres fabricated.

3.3. Percent Drug Content and Entrapment Efficiency

The drug content (DC) and entrapment efficiency (EE) data for different formulations are presented in Figure 3. Statistical analysis was conducted using ANOVA followed by Tukey's multiple comparisons tests. The results indicated a significant difference in the EE between ECSA1 and the remaining formulations ($p < 0.0001$), except for ECSA2, which showed a slightly lower but still significant difference ($p = 0.0008$). Further multiple comparisons revealed no significant difference in EE between formulations ECSA3 and ECSA5 ($p = 0.5294$) and between formulations ECSA6 and ECSAAg ($p = 0.3274$), respectively.

In terms of drug content, a comparison test showed that increasing the polymer content in a 2:1 (EC:SA) ratio did not lead to higher drug content, as observed between ECSA1 and ECSA6 ($p = 0.600$). Additionally, the inclusion of Ag NPs positively affected the drug content, as demonstrated by the significant difference between ECSA1 and ECSAAg ($p < 0.05$). A notable distinction exists between ECSA4 and the other formulations, with a p-value of < 0.0001, indicating statistical significance. This distinction can be primarily attributed to the considerable contrast in the polymer concentration, with ECSA4 exhibiting a significantly lower polymer concentration and higher drug content. Likewise, the results also demonstrate a significant difference between ECSA3 and the remaining formulations, primarily stemming from the lower initial drug content in ECSA3.

Table 2
Model application to the release profile data of different formulations of EC microspheres.

Kinetic Model	ECSA2	ECSA3	ECSA5	ECSA6	ECPSA
0th order					
R^2	0.9326	0.9228	0.92035	0.9022	0.9396
1st order					
R^2	0.6201	0.5879	0.5939	0.5738	0.5497
***K-P**					
R^2	0.9918	0.9941	0.9939	0.9896	0.9936
$k_k p$	5.3949	2.5412	7.1185	8.8608	0.6526
N	0.4887	0.5825	0.4932	0.4249	0.8572
***H-C**					
R^2	0.7770	0.7575	0.7535	0.7255	0.7745
k_X	0.0272	0.0256	0.0292	0.0254	0.0272
Higuchi					
R^2	0.9927	0.8878	0.9921	0.7755	0.2789
k_H	5.3612	2.6604	7.0920	8.4991	0.7958

*K-P:Korsmeyer-Peppas, **H-C:Hixon-Crowell

3.4. *In Vitro* Drug Release Assay

The calibration curve for SA is shown in Figure 4. It was formulated by taking different concentrations of SA and taking the absorbance reading at 305 nm. The *in vitro* release pattern for a time period of 96 hours is shown in Figure 5. ECSA6 shows a greater burst release despite having the same drug content as ECSA5 with a slight difference in the polymer content. Formulation ECSA5 showed the highest release percentage with a drug-polymer ratio of 1:1.

The burst release seen in other formulations was not seen when PEG was added into the internal phase of the fabrication process as seen in the release pattern of formulation ECPSA. In a 96-hour time frame, the release percentage does not exceed more than 63%. After 48 hours the release slows down and reaches a saturation point by day 5. Increasing the drug content as seen in formulation ECSA2 did not increase the amount of drug released from the microspheres. Formulation ECSA6 showed the highest cumulative release during the first 8 hours of the experiment while ECSA5 showed the highest release percentage. The study was designed to sustainably release SA from EC microspheres and data suggests that although the release is limited over such a long time period, the desired release pattern was achieved. All formulations showed sustained drug release when a T-test was done with a p-value < 0.05.Further modifications can either trigger quicker release or decrease the amount released from the microspheres. Further behavioral studies were done to objectively assess the fabricated microspheres as shown in Table 2. ECSA2 followed the Higuchi kinetic modeling drug dissolution pattern. The drug was released from the microspheres as a result of Fick's second law of diffusion, according to which an alteration in concentration can be identified at any given point in time [41, 42]. ECSA3, ECSA5, ECSA6, and ECPSA exhibited the Korsmeyer-Peppas drug-

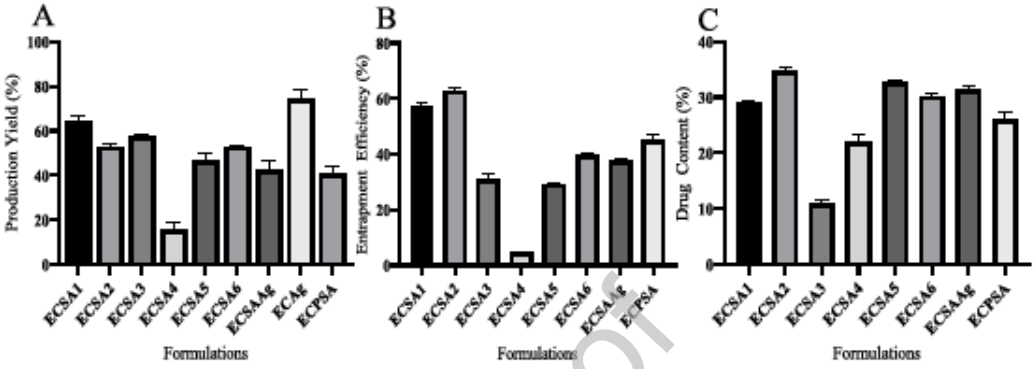
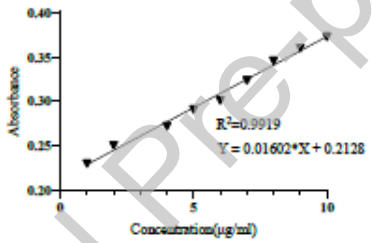
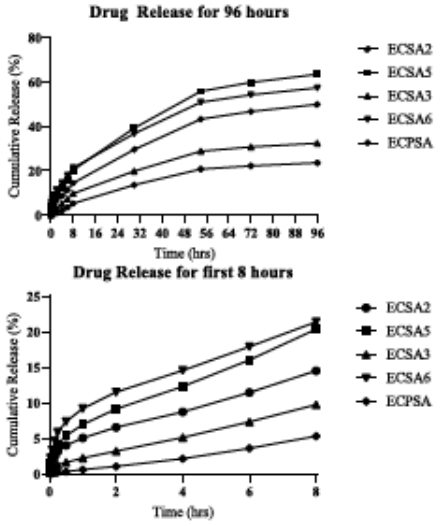


Figure 3: Graphical representation of: A. Production Yield, B. Entrapment efficiency of fabricated microparticles, C. Drug content of fabricated microparticles.



Curve Final.pdf

Figure 4: Calibration curve of Salicylic Acid (SA).



Release-Combined.pdf

Figure 5: *In vitro* drug release. Top: 96-hour drug release pattern; Bottom: 8-hour drug release pattern.

release kinetics model. The "N" value shows that ECSA6 followed Fick's law of diffusion (Case I diffusion), while ECSA3, ECSA5, and ECPSA showed Case II transport release mechanism (Non-Fickian diffusion) mechanism of drug release [43, 44, 45].

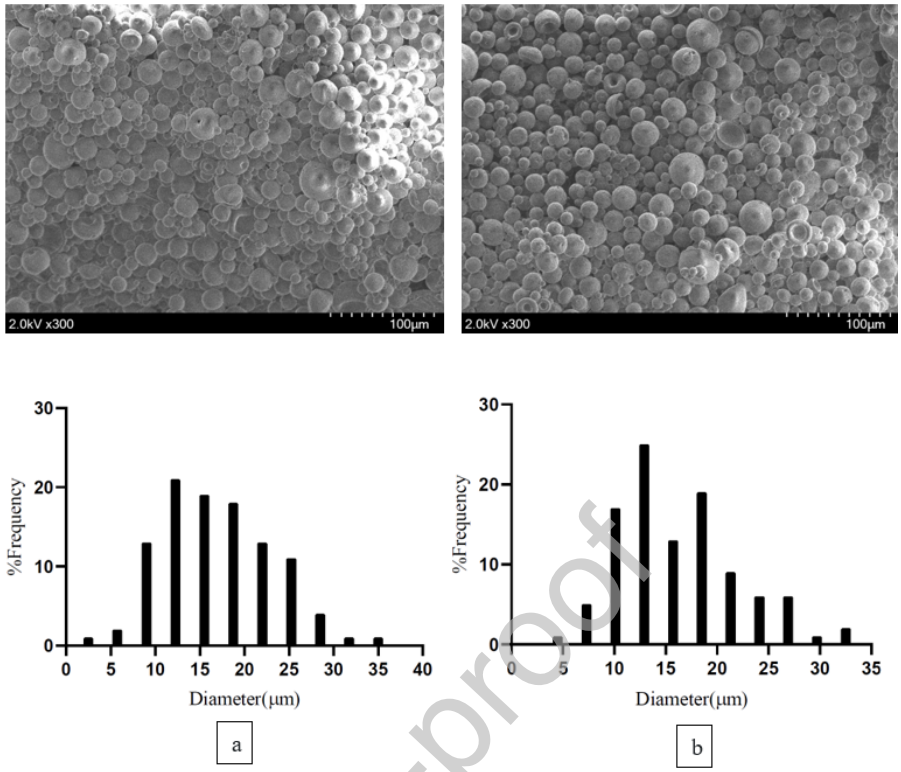
3.5. Particles Size Distribution

The particle size distribution for different formulations was analyzed. The particle size ranged from 5-40 μm for all formulations as shown in Table 3. The expected and desired size range was from 5-20 μm. Although the variation is high, the mean size range for all of the formulations was below 20 μm. The size is highly dependent on the stirring speed of the solution after the drop-wise addition of the internal phase.

For this study, all the formulations were stirred at the same speed of 700 rotations per minute (RPM), thereby yielding similar size particles. Using higher concentrations of EC and SA ensures that the PSD is broader as seen in Figure 6. Lower concentrations of polymer and drug resulted in a narrower PSD. This result could be attributed to the fact that there is a higher polymer content causing turbulence during spinning, subsequently leading to the fabrication of several microparticle sizes. Furthermore, the use of EC without SA results in a normalized distribution pattern as can be seen when particles were fabricated with Ag nanoparticles/drug and without the drug in Figure 7 (a) and (b). Silver nanoparticles did not cause any disruption in the formation of microspheres.

3.6. Micrometrics of Different Formulations

The micrometric results of the different formulations are shown in Table 4. Formulations ECSA1, ECSA2, ECSA5, and ECAg show good empirical powder flowability as the



and ECSA2.pdf

Figure 6: SEM images and size distributions (n=200) of particles produced by quasi-emulsion method (stirred for 22 hours at 700 RPM): (a) Formulation ECSA1 and (b) Formulation ECSA2.

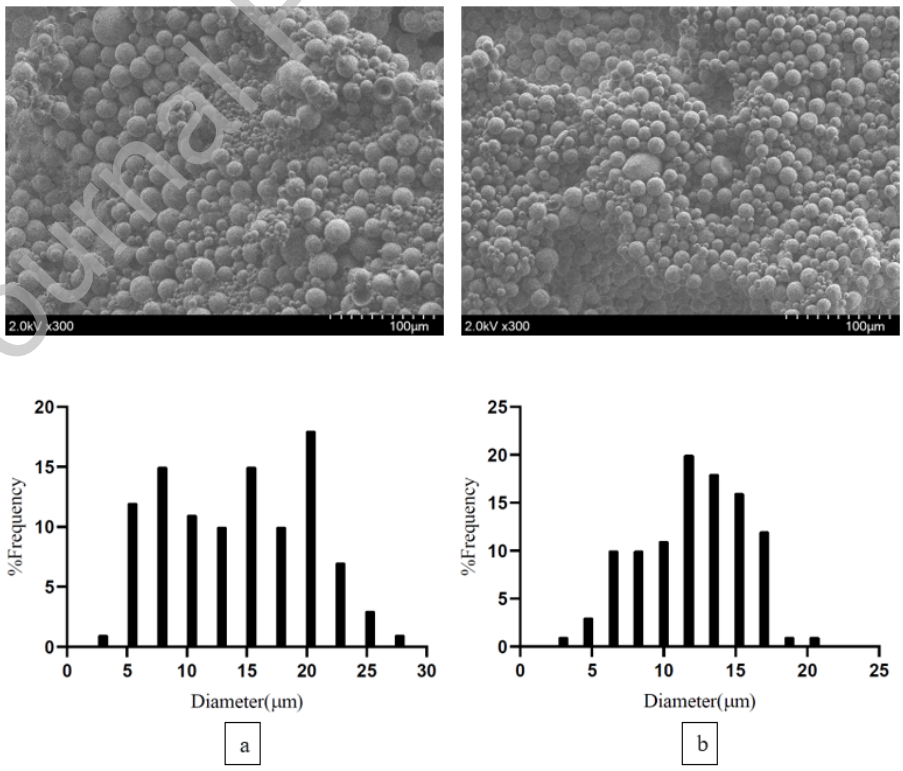


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Figure 7: SEM images and size distributions (n=200) of particles produced by quasi-emulsion method under the following conditions, rate per minute :700, stirred for 22 hours (a) Formulation ECSAAg (b) Formulation ECAg.

Table 3
Particle size distribution for different formulations used to fabricate microspheres.

Formulation	Min. (μm)	Max. (μm)	Mean & Std. Dev (μm)
ECSA1	2.12	34.12	15.42 ± 5.84
ECSA2	4.23	32.28	14.64 ± 5.56
ECSA3	5.29	32.96	17.75 ± 5.06
ECSA4	4.49	28.04	13.38 ± 4.26
ECSA5	3.17	23.33	10.15 ± 3.40
ECSA6	2.12	32.28	12.97 ± 4.86
ECSAAg	2.65	27.51	13.10 ± 5.92
ECAg	2.65	20.11	11.19 ± 3.57

Hausners ratio is lower than 1.18 [46]. If Carr's index is between 5 and 16%, this indicates good flow; if it is between 18 and 21%, this indicates fair flow; and if it is above 38%, this indicates very poor flow [47, 48].

As seen in Table 4, the microspheres fabricated using formulations ECSA1, ECSA2, and ECAg show good flow, while the rest indicate fair flow. The microspheres fabricated using formulation ECSA4 exhibit very poor flow as the concentration of the drug is higher than the polymer content. As indicated by the results a polymer-to-drug ratio of 2:1 had the best flowability. The flowability can be compromised by a negligible amount by considering the use of the formulation ECSA2 wherein the proportion of the drug is slightly higher. When Ag NPs are used in the fabrication process the flowability is good, but drastically reduces when SA is used in the mixture.

3.7. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of EC and PEG polymer, SA and two different formulations containing these materials are shown in Figure 8. FTIR spectra had the same peaks observed for different materials, highlighting the physio-chemical compatibility of the materials. Similar peaks show that the materials did not cause any unwanted reactions and were stable throughout the fabrication process. The C-O stretch of the carboxylic acid is attributed to the peaks at 1,049 cm^{-1} and 1,155 cm^{-1} for EC polymer. The C-H and O-H stretch were assigned to peaks at 2,868 cm^{-1} and 2,970 cm^{-1}respectively.

The FTIR spectra of SA show numerous peaks owing to its extensive structural variation. Vibrational peaks appeared at 657-754 cm^{-1} and were allocated to =C-H bending. C-OH phenolic stretching were attributed to peaks between 1,088, and 1,205 cm^{-1}. The strong phenolic stretching of SA is the reason for the C-O stretch of formulations ECSA1. For ECPSA the C-O stretch at 1,051 and 1,155 cm^{-1} is balanced by the O-H and C-O-H stretching assigned to peaks 1,094 and 1,241 cm^{-1}. Altogether, there is an alteration in the intensity and a slight shift in peaks attributed to C-H stretching in both of the formulations. Similarly, there is an alteration of peak intensity at 2,868 and 2,975 cm^{-1} attributed to C-H and O-H stretch respectfully, with the addition of SA as seen in Figure 8. Detailed analysis of the FTIR spectra of SA, PEG, EC, and formulations ECSA1, ECPSA is shown in Table 5. The obtained data of the FTIR spectra is well supported by literature [49, 50].

4. Conclusion and Future Direction

The fabrication of EC microspheres using the QESD method presents a highly efficient and cost-effective method for controlled drug release in diverse applications. In our study, we successfully synthesized EC microspheres combined with SA and Ag nanoparticles. Additionally, we explored the fabrication of microspheres using PEG and EC. The resulting microspheres exhibited a prolonged drug release profile. However, achieving the desired size and shape of the microspheres with minimal deviation proved challenging due to the employed fabrication technique. Nevertheless, the ease of fabrication and cost-effectiveness of this method establish a solid foundation for future investigations.

To enhance the capabilities of these microspheres, there are several avenues for further research. One aspect involves conducting drug release studies over more extended periods to assess the sustained release characteristics of the microspheres. Moreover, incorporating biodegradable polymers into the internal phase of the microspheres could enhance their porousness, leading to improved drug release profiles. Furthermore, the integration of Ag nanoparticles presents an exciting opportunity. The inclusion of Ag nanoparticles enables the utilization of near-infrared (NIR) light for expedited and targeted drug release from within the microspheres via thermal convection. This innovative approach harnesses the localized heating effect of NIR light, allowing for precise control and rapid release of drugs from the microspheres. The incorporation of this mechanism not only enhances the versatility of drug delivery but also opens up new possibilities for targeted therapies.

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6. Disclosure

The authors report no conflicts of interest in this work.

CRediT authorship contribution statement

Mishal Pokharel: Conceptualization of this study, Data Procurement and Analysis, Methodology, Writing, Editing. **Md Faiyaz Jamil:** Data Procurement and Analysis, Writing, Editing - Original draft preparation. **Jillian Pompei Wilson:** Editing - Original draft preparation. **Tracie Ferreira:** Editing - Original draft preparation. **Qinguo Fan:** Initiation of EC and SA study, Editing - Original draft preparation. **Kihan Park:** Writing, Editing - Original draft preparation.

Table 4
Micrometrics Results of different formulations of microsphere ECSA.

Formulations	Bulk Density (g/ml)	Tapped Density (g/ml)	Hausner's Ratio	Carr's Index(%)
ECSA1	0.345 ± 0.023	0.383 ± 0.053	1.084 ± 0.083	8.413 ± 0.083
ECSA2	0.362 ± 0.020	0.417 ± 0.040	1.142 ± 0.048	14.160 ± 0.048
ECSA3	0.294 ± 0.032	0.345 ± 0.023	1.192 ± 0.054	19.24 ± 0.005
ECSA4	1.211 ± 0.183	1.568 ± 0.143	1.303 ± 0.091	30.27 ± 0.009
ECSA5	0.289 ± 0.019	0.330 ± 0.026	1.179 ± 0.119	17.86 ± 0.119
ECSA6	0.306 ± 0.002	0.368 ± 0.053	1.231 ± 0.238	23.10 ± 0.238
ECSAAg	0.330 ± 0.026	0.425 ± 0.028	1.278 ± 0.048	27.78 ± 0.048
ECAg	0.28 ± 0.012	0.309 ± 0.017	1.096 ± 0.042	9.630 ± 0.042

Table 5
FTIR analysis of ingredients used in the formulation of microspheres SA and PEG and two formulations of microspheres: ECSA1 and ECPSA. **Abbreviations**: S-Strong, M-Medium, W-Weak

Peak observed (cm^{-1})	Strength	Functional group
FTIR of EC		
1,049, 1,155	W	C-O stretch in carboxylic acid, ester, alcohol and ether (1,320âĂŞ1,000 cm-1)
1,353, 1,373	M	C-H stretch in plane bed (1,430âĂŞ1,290 cm-1)
2,868, 2,970	W	C-H stretch (3,000âĂŞ2,850 cm-1) and O-H stretch (3,300âĂŞ2,500 cm-1)
FTIR of SA		
656-754	S	=C-H bending
1,088-1,205	M-W	C-OH phenolic stretching
1,289	M	C-O in carboxylic acid stretching
1,323	W	O-H phenolic bending
1,380, 1,440, 1,653	M	C=O asymmetric and symmetric carboxylic (COO) bond stretching
1,559, 1,576	M-W	C=C phenolic multiple peaks
1,405-1,479	M-W	C-C stretching
3,233, 2,830	M	O-H stretch (3,300âĂŞ2,500 cm-1)
FTIR of PEG		
1,094, 1,241	S-M	O-H and C-O-H stretching
2,876	M	C-H stretching
1,342, 1,466	W	C-H bending
FTIR of ECSA1		
1,051, 1,155	S	C-O stretch in carboxylic acid, ester, alcohol and ether
1,309, 1,356, 1,373	M	-H stretch in plane bed
2,868,2975	M	C-H stretch and O-H stretch
FTIR of ECPSA		
1,051, 1,155	M-W	C-O stretch in carboxylic acid, ester, alcohol and ether
1,353, 1,373	W	C-H stretch in plane bed
2,868,2,972	W	C-H stretch and O-H stretch

References

[1] K. Rama, P. Senapati, M. Das, Formulation and in vitro evaluation of ethyl cellulose microspheres containing zidovudine, Journal of microencapsulation 22 (8) (2005) 863–876.

[2] J. I. Pérez-Martínez, E. Morillo, C. Maqueda, J. M. Gines, Ethyl cellulose polymer microspheres for controlled release of norfluazon, Pest Management Science: Formerly Pesticide Science 57 (8) (2001) 688–694.

[3] S. Sahoo, A. Mallick, B. Barik, P. Senapati, Preparation and in vitro evaluation of ethyl cellulose microspheres containing stavudine by the double emulsion method, Die Pharmazie-An International Journal of Pharmaceutical Sciences 62 (2) (2007) 117–121.

[4] T.-L. WU, W-J LIN, Modification of the initial release of a highly water-soluble drug from ethyl cellulose microspheres, Journal of Microencapsulation 16 (5) (1999) 639–646.

[5] S.-J. Cheu, R.-L. Chen, P.-F. Chen, W.-J. Lin, In vitro modified release of acyclovir from ethyl cellulose microspheres, Journal of microencapsulation 18 (5) (2001) 559–565.

[6] K. Sarkar, S. M. A. Sadat, M. S. Islam, R.-u. Jalil, Study of ethyl cellulose based sustained release microspheres of naproxen sodium, Dhaka University Journal of Pharmaceutical Sciences 10 (2) (2011) 123–129.

[7] V. Saez, J. M. Freitas, J. R. Hernández, C. Regina Elias Mansur, Val-

Short Title of the Article

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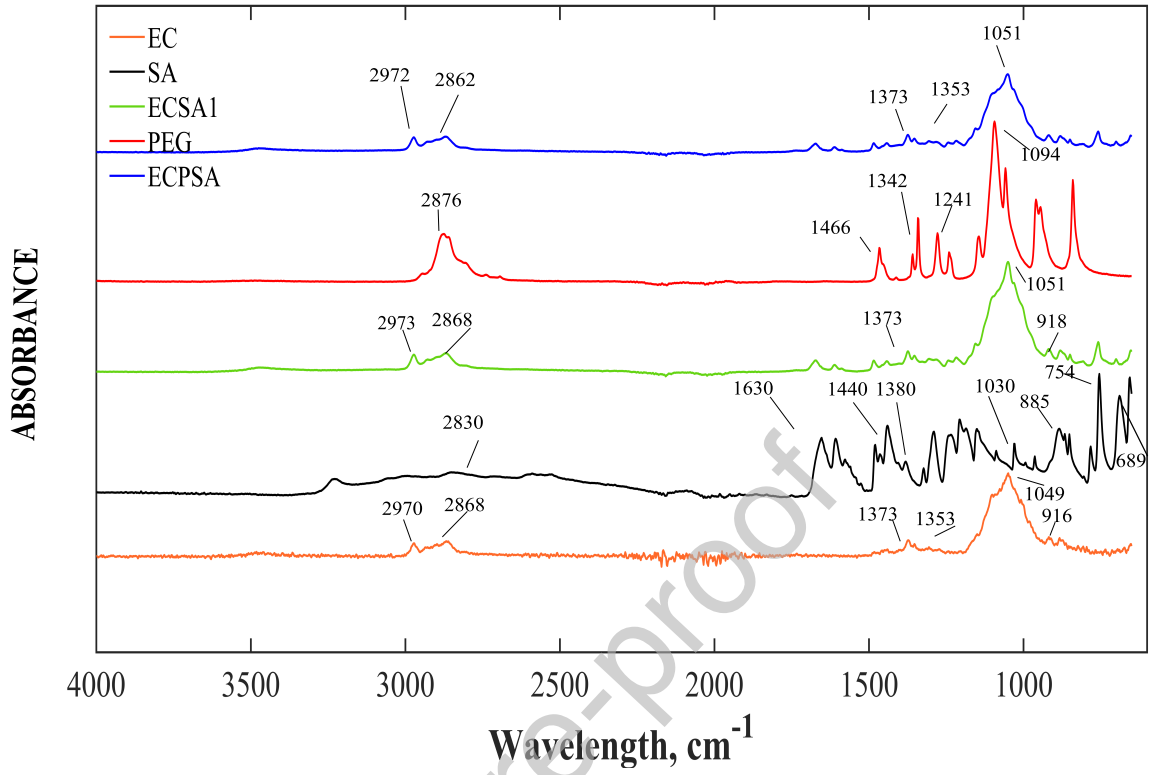

Figure 8: FTIR graph with labeled peaks for EC,ECSA1, PEG, ECPSA and SA.

idation of uv spectrophotometric method for quantifying ketoconazole encapsulated in ethyl cellulose microspheres, in: Macromolecular Symposia, Vol. 380, Wiley Online Library, 2018, p. 1800066.

[8] N. Chella, K. K. Yada, R. Vempati, Preparation and evaluation of ethyl cellulose microspheres containing diclofenac sodium by novel w/o/o emulsion method, Journal of Pharmaceutical Sciences and Research 2 (12) (2010) 884.

[9] A. C. Capomacchia, M. Thakare, S. T. Graner, H. Ahmed, P. Graner, D. Elder, J. C. Price, B. Israel, Formulation parameters and release mechanism of theophylline loaded ethyl cellulose microspheres.

[10] M. Thakare, B. Israel, S. T. Garner, H. Ahmed, P. Garner, D. Elder, J. C. Price, A. C. Capomacchia, Formulation parameters and release mechanism of theophylline loaded ethyl cellulose microspheres: effect of different dual surfactant ratios, Pharmaceutical development and technology 18 (5) (2013) 1213–1219.

[11] A. Zafar, M. Afzal, A. M. Quazi, M. Yasir, I. Kazmi, F. A. Al-Abaasi, N. K. Alruwaili, K. S. Alharbi, S. I. Alzarea, S. Sharma, et al., Chitosan-ethyl cellulose microspheres of domperidone for nasal delivery: Preparation, in-vitro characterization, in-vivo study for pharmacokinetic evaluation and bioavailability enhancement, Journal of Drug Delivery Science and Technology 63 (2021) 102471.

[12] H. Feng, L. Zhang, C. Zhu, Genipin crosslinked ethyl cellulose–chitosan complex microspheres for anti-tuberculosis delivery, Colloids and Surfaces B: Biointerfaces 103 (2013) 530–537.

[13] W. Cai, H. Yang, D. Han, X. Guo, A physical route to porous ethyl cellulose microspheres loaded with tio2 nanoparticles, Journal of Applied Polymer Science 131 (19).

[14] P. Shi, Y. Li, L. Zhang, Fabrication and property of chitosan film carrying ethyl cellulose microspheres, Carbohydrate polymers 72 (3) (2008) 490–499.

[15] P. Shi, Y. Zuo, Q. Zou, J. Shen, L. Zhang, Y. Li, Y. S. Morsi, Improved properties of incorporated chitosan film with ethyl cellulose microspheres for controlled release, International Journal of Pharmaceutics 375 (1-2) (2009) 67–74.

[16] H. Liu, L. Zhang, P. Shi, Q. Zou, Y. Zuo, Y. Li, Hydroxyapatite/polyurethane scaffold incorporated with drug-loaded ethyl cellulose microspheres for bone regeneration, Journal of Biomedical Materials Research Part B: Applied Biomaterials 95 (1) (2010) 36–46.

[17] P. Ahmadi, A. Jahanban-Esfahlan, A. Ahmadi, M. Tabibiazar, M. Mohammadifar, Development of ethyl cellulose-based formulations: A perspective on the novel technical methods, Food Reviews International 38 (4) (2022) 685–732.

[18] T. Miyamoto, S.-i. Takahashi, H. Ito, H. Inagaki, Y. Noishiki, Tissue biocompatibility of cellulose and its derivatives, Journal of biomedical materials research 23 (1) (1989) 125–133.

[19] B. Tian, S. Tang, Y. Li, T. Long, X.-H. Qu, D.-G. Yu, Y.-J. Guo, Y.-P. Guo, Z.-A. Zhu, Fabrication, characterization, and biocompatibility of ethyl cellulose/carbonated hydroxyapatite composite coatings on ti6al4v, Journal of Materials Science: Materials in Medicine 25 (2014) 2059–2068.

[20] J. Rao, C. Shen, Z. Yang, O. A. Fawole, J. Li, D. Wu, K. Chen, Facile microfluidic fabrication and characterization of ethyl cellulose/pvp films with neatly arranged fibers, Carbohydrate Polymers 292 (2022) 119702.

[21] M. E. Reyes-Melo, I. Y. Miranda-Valdez, J. G. Puente-Córdova, C. A. Camarillo-Hernández, B. López-Walle, Fabrication and characterization of a biocompatible hybrid film based on silver nanoparticle/ethyl cellulose polymer, Cellulose 28 (2021) 9227–9240.

[22] E. Kondolot Solak, S. Kaya, G. Asman, Preparation, characterization, and antibacterial properties of biocompatible material for wound heal-

ing, Journal of Macromolecular Science, Part A 58 (10) (2021) 709–716.

[23] X.-C. Song, Y.-L. Yu, G.-Y. Yang, A.-L. Jiang, Y.-j. Ruan, S.-h. Fan, One-step emulsification for controllable preparation of ethyl cellulose microcapsules and their sustained release performance, Colloids and Surfaces B: Biointerfaces 216 (2022) 112560.

[24] P. Randjelović, S. Veljković, N. Stojiljković, D. Sokolović, I. Ilić, D. Laketić, D. Randjelović, N. Randjelović, The beneficial biological properties of salicylic acid, Acta Fac. Med. Naissensis 32 (4) (2015) 259–265.

[25] M. T. Ruffin IV, D. Normolle, M. A. Vaerten, M. Peters-Golden, D. E. Brenner, K. Krishnan, C. L. Rock, C. R. Boland, J. Crowell, G. Kelloff, Suppression of human colorectal mucosal prostaglandins: determining the lowest effective aspirin dose, Journal of the National Cancer Institute 89 (15) (1997) 1152–1160.

[26] J. Paterson, J. Lawrence, Salicylic acid: a link between aspirin, diet and the prevention of colorectal cancer, Qjm 94 (8) (2001) 445–448.

[27] G. Levy, Pharmacokinetics of salicylate elimination in man, Journal of pharmaceutical sciences 54 (7) (1965) 959–967.

[28] S. Mathurkar, P. Singh, K. Kongara, P. Chambers, Pharmacokinetics of salicylic acid following intravenous and oral administration of sodium salicylate in sheep, Animals 8 (7) (2018) 122.

[29] M. Á. Cabrera-Pérez, H. Pham-The, M. F. Cervera, R. Hernández-Armengol, C. Miranda-Pérez de Alejo, Y. Brito-Ferrer, Integrating theoretical and experimental permeability estimations for provisional biopharmaceutical classification: Application to the who essential medicines, Biopharmaceutics & Drug Disposition 39 (7) (2018) 354–368.

[30] G. L. Amidon, H. Lennernäs, V. P. Shah, J. R. Crison, A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability, Pharmaceutical research 12 (1995) 413–420.

[31] J. B. Dressman, A. Nair, B. Abrahamsson, D. M. Barends, D. Groot, S. Kopp, P. Langguth, J. E. Polli, V. P. Shah, M. Zimmer, Biowaiver monograph for immediate-release solid oral dosage forms: acetylsalicylic acid, Journal of pharmaceutical sciences 101 (8) (2012) 2653–2667.

[32] A. Kornhauser, S. G. Coelho, V. J. Hearing, Applications of hydroxy acids: classification, mechanisms, and photoactivity, Clinical, cosmetic and investigational dermatology (2010) 135–142.

[33] A. N. Lin MD, FRCPC, T. Nakatsui MD, Salicylic acid revisited, International journal of dermatology 37 (5) (1998) 335–342.

[34] Z. Draelos, Rediscovering the cutaneous benefits of salicylic acid, Cosm Derm 10 (Suppl 4) (1997) 4.

[35] M. Rajkumar, K. Lee, H. Freitas, Effects of chitin and salicylic acid on biological control activity of pseudomonas spp. against damping off of pepper, South African Journal of Botany 74 (2) (2008) 268–273.

[36] S. T. Schenk, A. Schikora, Ahl-priming functions via oxylipin and salicylic acid, Frontiers in Plant Science 5 (2015) 784.

[37] G. De Marco, S. Afsa, M. Galati, G. Guerriero, A. Mauceri, H. Ben Mansour, T. Cappello, Time-and dose-dependent biological effects of a sub-chronic exposure to realistic doses of salicylic acid in the gills of mussel mytilus galloprovincialis, Environmental Science and Pollution Research 29 (58) (2022) 88161–88171.

[38] D. Dempsey, D. F. Klessig, How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans?, BMC biology 15 (1) (2017) 1–11.

[39] J. R. Vane, Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs, Nature new biology 231 (1971) 232–235.

[40] R. K. Madan, J. Levitt, A review of toxicity from topical salicylic acid preparations, Journal of the American Academy of Dermatology 70 (4) (2014) 788–792.

[41] D. Solanki, M. Motiwale, Studies on drug release kinetics and mechanism from sustained release matrix tablets of isoniazid using natural polymer obtained fromdioscorea alata, Int J ChemTech Res 13 (2020) 166–73.

[42] I. Permanadewi, A. Kumoro, D. Wardhani, N. Aryanti, Modelling of controlled drug release in gastrointestinal tract simulation, in: Journal of Physics: Conference Series, Vol. 1295, IOP Publishing, 2019, p. 012063.

[43] L. Ahmed, R. Atif, T. S. Eldeen, I. Yahya, A. Omara, M. Eltayeb, Study the using of nanoparticles as drug delivery system based on mathematical models for controlled release, Int. J. Eng. Technol 8 (2019) 52–56.

[44] T. S. P. Cellet, G. M. Pereira, E. C. Muniz, R. Silva, A. F. Rubira, Hydroxyapatite nanowhiskers embedded in chondroitin sulfate microspheres as colon targeted drug delivery systems, Journal of Materials Chemistry B 3 (33) (2015) 6837–6846.

[45] J. M. Unagolla, A. C. Jayasuriya, Drug transport mechanisms and in vitro release kinetics of vancomycin encapsulated chitosan-alginate polyelectrolyte microparticles as a controlled drug delivery system, European Journal of Pharmaceutical Sciences 114 (2018) 199–209.

[46] T. Hao, Understanding empirical powder flowability criteria scaled by hausner ratio or carr index with the analogous viscosity concept, RSC advances 5 (70) (2015) 57212–57215.

[47] N. Yüksel, B. Türkmen, A. KURDOĞLU, B. BAŞARAN, J. Erkin, T. Baykara, Lubricant efficiency of magnesium stearate in direct compressible powder mixtures comprising cellactose® 80 and pyridoxine hydrochloride, Fabad Journal of Pharmaceutical Sciences 4 (32) (2007) 173–183.

[48] A. Moghbel, H. Abbaspour, Study of compressibility properties of yogurt powder in order to prepare a complementary formulation, Iranian Journal of Pharmaceutical Research: IJPR 12 (3) (2013) 231.

[49] A. Kantouch, A. A. El-Sayed, M. Salama, A. Abou El-Kheir, S. Mowafi, Salicylic acid and some of its derivatives as antibacterial agents for viscose fabric, International journal of biological macromolecules 62 (2013) 603–607.

[50] M. Trivedi, A. Branton, D. Trivedi, H. Shettigar, K. Bairwa, S. Jana, Fourier transform infrared and ultraviolet-visible spectroscopic characterization of biofield treated salicylic acid and sparfloxacin, Natural Products Chemistry & Research 5 (3).

Conflict of Interest

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: