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ORIGINAL ARTICLE

Preparation and Characterization of the Release Behavior of PVA: Na-Alg Microsphere Containing Fampridine

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ABSTRACT

Objectives: This study focuses on both the formulation of bio-based microspheres containing fampridine for the treatment of multiple sclerosis and provides an alternative to the commercially available product (Fampyra 10 mg, Biogen).

Materials and Methods: The encapsulation of fampridine was achieved using polyvinyl alcohol (PVA) and sodium alginate (Na-Alg) polymers. Glutaraldehyde (GA) and hydrochloric acid (HCl) were used as crosslinking agents. The polymer ratio (PVA: Na-Alg), drug: polymer (*d:p*) ratio, cross-linking agent ratio, and cross-linking time were evaluated for fampridine release. Release studies were analyzed using an ultraviolet spectrophotometer. The microspheres were characterized using scanning electron microscopy, differential scanning calorimetry, and Fourier transform-infrared transform infrared spectroscopy (FT-IR). The particle sizes of the fampridine-loaded microspheres were determined using a laser light scattering device.

Results: The study revealed that the optimal conditions for achieving the highest fampridine release involved microspheres formulated with a polymer ratio of PVA:Na-Alg (*w:w*) at 1:1, a drug-to-polymer (*d:p*) ratio of 1:2 (*w:w*), cross-linking agent concentrations of 2.5% (*w:w*) GA and 3% (*w:w*) HCl, and a cross-linking time of 5 minutes. The particle size analysis showed that all microspheres were within the 300-800 µm range, and an increase in the *d:p* ratio correlated with larger particle sizes.

Conclusion: The findings demonstrate that bio-based microspheres containing fampridine can be successfully formulated using PVA and sodium alginate polymers, providing a promising alternative to the commercially available product..

Keywords: Drug release, fampridine, microsphere, PVA: Na-Alg.

INTRODUCTION

Multiple sclerosis (MS) is a persistent condition characterized by inflammation and myelin loss, which affects the myelinated nerve fibers within the central nervous system. The components employed in the treatment of MS are glatiramer acetate, dimethyl fumarate, fingolimod, teriflunomide, natalizumab, alemtuzumab, mitoxantrone, fampridine, ocrelizumab.[1-5] Fampridine's performance-based assessments have demonstrated its ability to enhance walking speed, motor control, and balance in approximately 40% of patients who receive treatment.[6,7] In the treatment of MS, even though many molecules are used in various segments, the extended-release 10 mg fampridine tablet, taken twice daily, is the only approved pharmacological drug for improving walking ability in adults with MS.[8] Injectable hydrogels, direct implantation, crosslinked micelles, and injectable suspensions have been used in drug delivery systems.[9-12] Biodegradable polymers and crosslinking agents may offer advantages.[13-15]

Due to sodium alginate's (Na-Alg) biologically degraded, strong gel-forming characteristics, and cost-effectiveness, Na-Alg has been extensively used in drug delivery systems.[16] All polyvinyl alcohol (PVA): Na-Alg hydrogels are pH sensitive, and using a lower cross-linking agent leads to a higher swelling ratio.[17] The commonly used crosslinking agent combination for PVA: Na-Alg hydrogels is glutaraldehyde (GA) and hydrochloric acid (HCl).[18,19]

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Na-Alg also improved the encapsulation efficiency (EE) and particle size effect of microspheres, and the single-step polymerization process resulted in a narrow particle size distribution.[20-22]

The results revealed that the applied extraction model increased the initial burst effect and reduced the EE.[23]

Indeed, in microspheres, particle size is directly associated with EE and drug release. Studies have reported that as the polymer ratio, cross-linking ratio, and cross-linking time increase, the particle size tends to increase.[24]

In this study, the effects of various processing parameters on fampridine release from PVA: Na-Alg microspheres were examined for the treatment of MS.

MATERIALS AND METHODS

Materials

Chemicals

Fampridine (Enaltec, India), PVA (M_A = 72,000 g/mole) (Merck, Germany), Na-Alg (M_A = 8,000-12,000 g/mole) (Merck, Germany), GA (Merck, Germany), HCl (Merck, Germany), Na_2HPO_4 (Merck, Germany), NaH_2PO_4 (Merck, Germany), Methanol (Merck, Germany), NaOH (Merck, Germany), and Acetic Acid (Merck, Germany).

Equipments

Peristaltic pump (150 mL/h flow rate) (Lefoo, China), Ultraviolet (UV)2-100 UV/visible spectrophotometer (Unicam, Netherlands), Shaking Water Bath (Mettler Toledo, Switzerland), Magnetic Stirrer (Chiltren, United Kingdom), Incubator (Memmert, Germany), pH Meter (Mettler Toledo, Switzerland), Analytical Scales (Mettler Toledo, Switzerland). FT-IR (Mattson, ABD), scanning electron microscopy (SEM) (JSM 5600, Japan), differential scanning calorimetry (DSC) (Dupont 2000), Mastersizer 2000 instrument (Malverm Instruments, UK), Filter paper whatman number: 42 (Merck, Germany).

Methods

Preparation of the PVA: Na-Alg microspheres

The formulations of the PVA: Na-Alg and empty microspheres are shown in Tables 1 and 2, respectively. Microspheres were obtained using the liquid maturation method. Fampridine was added to the polymer blends and stirred for approximately 12 hours to form a suspension. Then, these suspensions were passed through a peristaltic pump and dripped as droplets at a flow rate of 150 mL/h from a height of 3 cm into solutions of 50 mL HCl and GA, respectively. The formation of microspheres with different PVA: Na-Alg compositions was conducted at room temperature. The transfer of 20 mL drug-polymer mixture was completed in 8-10 minutes. After the last drop,

Table 1. Formulation of microspheres

	PVA: Na-Alg	Cross-linking agent concentration (*v:v*)	Cross-linking time (min.)	Drug: Polymer ratio
O1	1:1	2.5% GA + 3GA+ 3% HCl	5	1:0.5
O2	1:1	2.5% GA + 3% HCl	5	1:1
O3	1:1	2.5% GA + 3% HCl	5	1:1.25
O4	1:1	2.5% GA + 3% HCl	5	1:1.5
O5	1:1	2.5% GA + 3% HCl	5	1:2
P1	2:1	2.5% GA + 3% HCl	5	1:0.5
P2	2:1	2.5% GA + 3% HCl	5	1:1
P3	2:1	2.5% GA + 3% HCl	5	1:1.25
P4	2:1	2.5% GA + 3% HCl	5	1:1.5
P5	2:1	2.5% GA + 3% HCl	5	1:2
K1	3:1	2.5% GA + 3% HCl	5	1:0.5
K2	3:1	2.5% GA + 3% HCl	5	1:1
K3	3:1	2.5% GA + 3% HCl	5	1:1.25
K4	3:1	2.5% GA + 3% HCl	5	1:1.5
K5	3:1	2.5% GA + 3% HCl	5	1:2
L1	3:1	2.5% GA + 3% HCl	2.5	1:1.25
L2	3:1	2.5% GA + 3% HCl	30	1:1.25
M1	3:1	3% GA + 3% HCl	5	1:1.25
M2	3:1	1.5% GA + 1.5% HCl	5	1:1.25

PVA: Polyvinyl alcohol, Na-Alg: Sodium alginate, GA: Glutaraldehyde, HCl: Hydrochloric acid, min.: Minute

Table 2. Formulation of empty microspheres

	PVA: Na-Alg	Cross-linking agent concentration (*v:v*)	Cross-linking time (min.)
O	1:1	2.5% GA+ 3% HCl	5
P	2:1	2.5% GA+ 3% HCl	5
K	3:1	2.5% GA+ 3% HCl	5

PVA: Polyvinyl alcohol, Na-Alg: Sodium alginate, GA: Glutaraldehyde, HCl: Hydrochloric acid, min.: Minute

the microspheres were matured by stirring in the cross-linking solution (GA + HCl) for 2.5, 5, and 30 min. The microspheres were then washed with distilled water and filtered through a filter paper.

Release studies of fampridine from PVA: Na-Alg microspheres

25 mg microsphere samples were placed in 250 mL of buffer solutions within 500 mL bottles and positioned in a shaking water bath. Release studies were conducted at 37 ˚C in pH 1.2 HCl solution, pH 6.8, and pH 7.4. The microspheres were filtered every two hours, and the next buffer solution was introduced. Four mL samples were collected from the solution, and absorbance values at 262 nm were determined using a UV spectrophotometer. Concentrations were calculated using calibration graph shown in Figure 1 based on standard fampridine solutions. All release studies were conducted with 6 samples (n= 6) and average fampridine release was used as a result.

Analytical method

The calibration graph of fampridine is shown in Figure 1. The standard solutions were prepared from 0.1 to 2.5 mmol/L. All absorbance values were read thrice, and the mean value was used.

Analytical method validation

The selectivity, linearity, range, system precision, and repeatability of the relevant method have been assessed.[25] All absorbance values were read thrice, and the mean value was used.

Selectivity

UV spectra at 262 nm were obtained for all excipients present in the microsphere content (HCl, GA, PVA, Na-Alg). The possibility of interference with fampridine at the same wavelength was evaluated.

Linearity and range

The absorbance values were obtained using standard fampridine solutions at five different concentrations (0.5 mmol/L, 0.8 mmol/L, 1.0 mmol/L, 2.0 mmol/L, 2.5 mmol/L). The results were evaluated, and a linearity graph was generated.

System precision

The standard fampridine solution was prepared at a concentration of 1 mmol/L and was read 6 times using a UV spectrophotometer, and the standard deviation (SD) was measured.

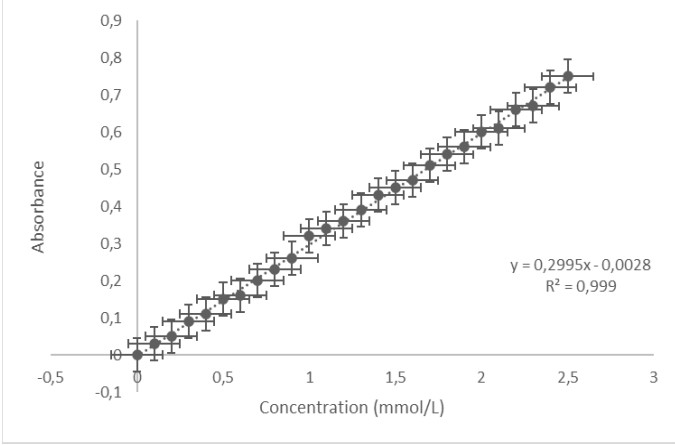

Figure 1. Calibration graph of fampridine

Repeatability

Microspheres labeled O5 were prepared six times (n= 6) on two different days, and their absorbance values were recorded at 262 nm. The SD was measured.

The water content of microspheres

50 mg microspheres were immersed in 100 mL of distilled water for a 24-hour equilibration period. Equilibrium water content was calculated using Equation 1.

$$\text{The equilibrium water content } \% = \frac{Wf - Wd}{Wd} \text{ x } 100 \qquad \text{[Eq. 1]}$$

Wf shows the microsphere weight, which includes water, and Wd shows the dry microsphere weight.

Encapsulation Efficiency (EE) of microspheres

50 mg of microspheres containing fampridine were pulverized in an agate mortar and extracted in 50 mL of methanol under cooling for 4 h. The obtained extract was then filtered, and diluted to various concentrations, and the drug content was quantified using UV spectrophotometry. The EE is calculated using Equation 2.

$$EE\ \% = \frac{D}{T} \times 100 \qquad \text{[Eq. 2]}$$

D shows the actual amount of drug in the microspheres, and T shows the theoretical amount of drug in the microspheres.

RESULTS

Effect of PVA: Na-Alg ratio on fampridine release

O3, P3, and K3 were selected with all other parameters kept constant, solely varying the PVA: Na-Alg ratio. Results are shown in Figure 2. Entering PVA into the formulation leads to an increase in hydrogen bonding interactions between PVA and Na-Alg, and due to the functional group interactions between Na-Alg and fampridine, it was observed that the amount of PVA increased, the release of fampridine decreased, and the diffusion of the solution into the polymeric microsphere more challenging.[26,27]

Effect of pH on fampridine release

Fampridine release was conducted at pH 1.2, 6.8, and 7.4 for 14 h. The results showed that all microspheres are pH-sensitive.[28] There was maximum release at pH 7.4 (89% after 18 hours), decreasing release at pH 6.8 (73% after 18 hours), and the lowest release occurred at pH 1.2 (67% after 18 hours). This is attributed to the low ionization percentage of fampridine at low pH. Additionally, in controlled release systems containing PVA, the best swelling is observed at high pH, which is consistent with the swelling results obtained from release studies.[28,29] Because the highest release was observed at pH 7.4, subsequent experiments were conducted using a pH 7.4 buffer solution as the medium.

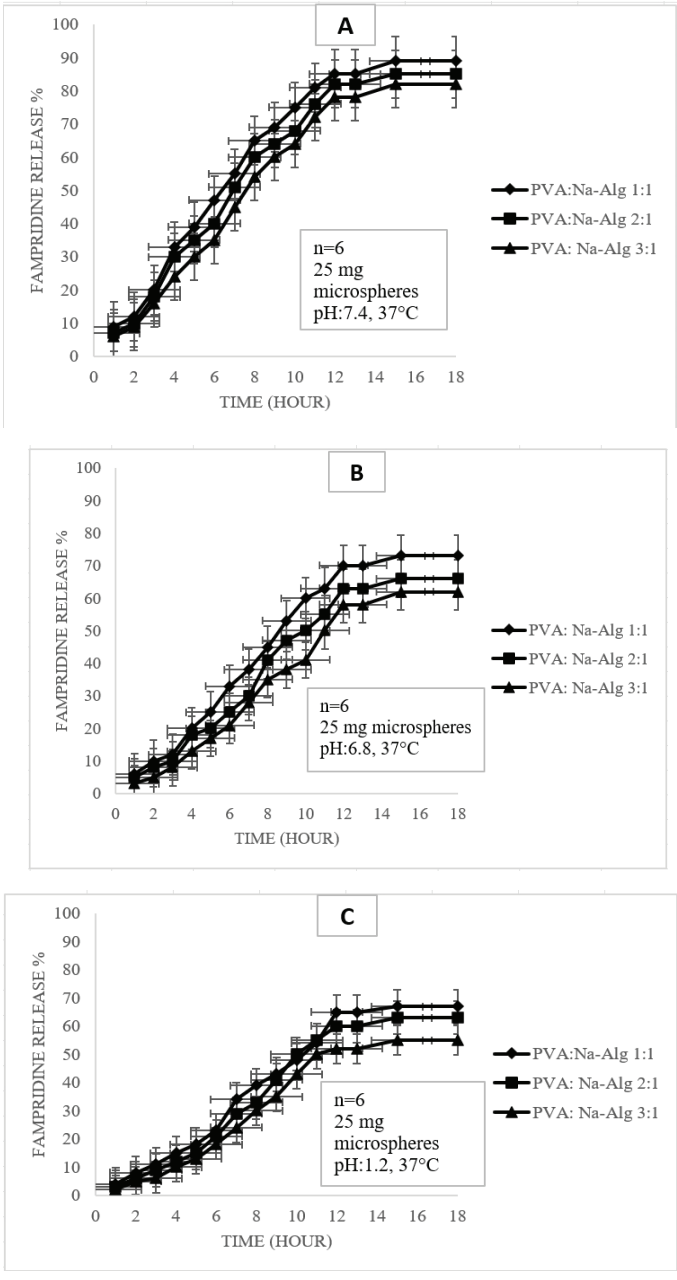

Figure 2. Effect of PVA: Na-Alg ratio. A): pH: 7.4, B): pH: 6.8, C): pH: 1.2
PVA: Polyvinyl alcohol, Na-Alg: Sodium alginate

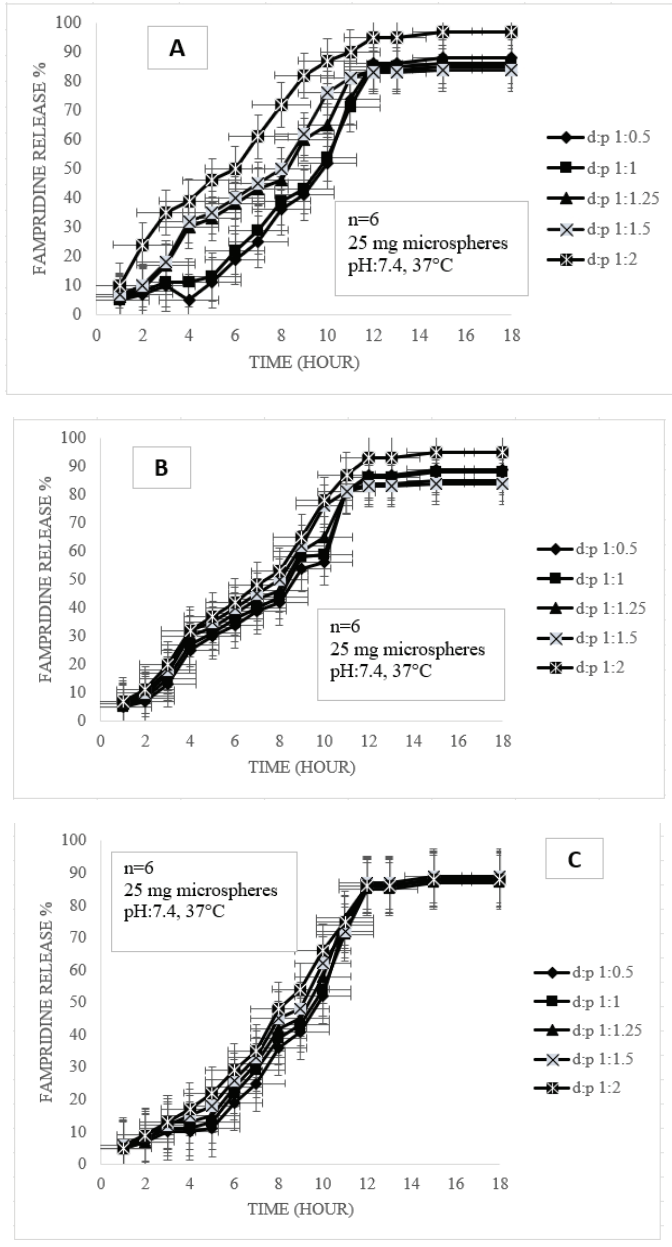

Figure 3. Effect of the drug: polymer ratio. A): PVA:Na-Alg (1:1), B): PVA: Na-Alg (2:1), C):
PVA: Na-Alg (3:1) PVA: Polyvinyl alcohol, Na-Alg: Sodium alginate

Effect of the drug: polymer ratio on fampridine release

O1-K5 was used to evaluate the drug: polymer ratio, and the results are shown in Figure 3. It was observed that the drug: polymer ratio decreased and the release of fampridine increased.

The highest release of fampridine was observed with a drug: polymer ratio of 1:2. These results can be explained by the microspheres adopting a more compact structure as the drug quantity increases, making it more difficult for the solvent to permeate the microsphere matrix. Increasing the drug quantity leads to a deceleration in drug release due to increased interactions between the drug and polymer blends.[30]

Effect of cross-linking agent concentration on fampridine release

The microspheres were prepared using three different cross-linking agent concentrations: 3% GA + 3% HCl, 2.5% GA + 3% HCl, 1.5% GA + 1.5% HCl, and K3, M1, and M2 were analyzed. The results are shown in Figure 4. Increasing the concentration of the cross-linking agent in the microspheres leads to less diffusion of the solvent into the microspheres and made more challenging of drug diffusion.[31,32]

Effect of cross-linking time on fampridine release

2.5, 5, and 30 min were assessed with K3, L1 and L2. Results are shown in Figure 5. It was observed that the highest fampridine release occurred at a low crosslinking time. But there is not a significant difference between 2.5 and 5 min. It was observed that the cross-linking time increased, leading to a reduction in polymer chain mobility and less solvent diffusion into the microspheres.[32]

The water content of microspheres

Results are shown in Table 3. It was observed that the highest swelling occurred at pH 7.4. This is due to the low swelling of Na-Alg and PVA in acidic environments in the structure of the microspheres.[28]

EE and particle size of microspheres

The encapsulation efficiencies are listed in Table 4. Results showed that the EE increased parallelly with the drug: polymer ratio. At a constant drug: polymer ratio, an increase in the PVA: Na-Alg ratio was observed to increase the EE. This result is attributed to the increased amount of polymer in the structure, leading to the diffusion of more drugs into the polymer.

The particle sizes of the microspheres were between 300 and 800 µm. Increasing amounts of fampridine and PVA led to an increase in particle size because of the decrease in the cross-linking density.[24,33,34]

Analytical method validation

Selectiviy

Because the absorbance values of PVA, GA, and HCl at 262 nm are all zero, it is possible to conclude that the method was selective for fampridine.

Linearity and range

Results are shown in Figure 6 and Table 5. The results demonstrate that the proposed method is linear and within the range (R^2 = 0.999).

System precision

System precision results are shown in Table 6. The results are remarkably close to each other, and the SD value is quite low. It can be concluded that the proposed system provides accurate and precise results.

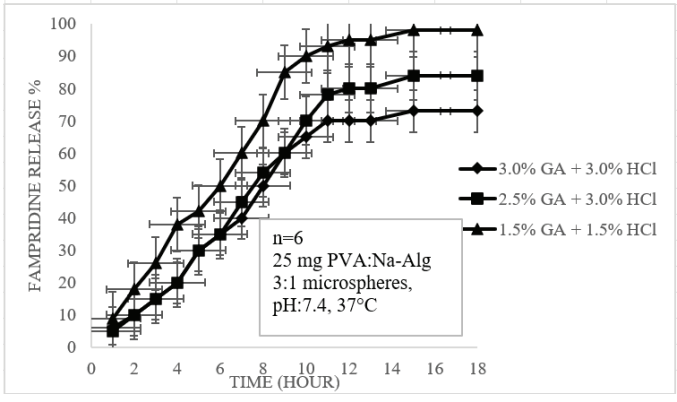

Figure 4. Effect of cross-linking agent concentration
PVA: Polyvinyl alcohol, Na-Alg: Sodium alginate

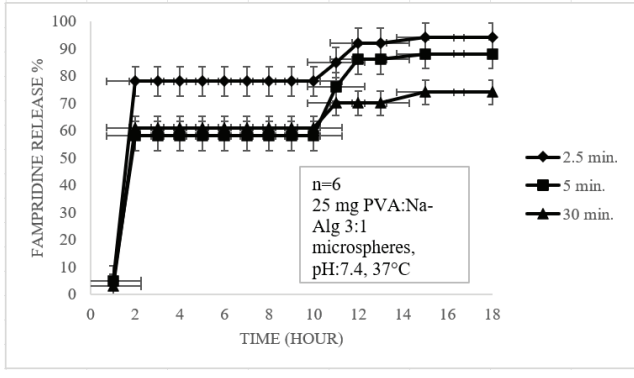

Figure 5. Effect of cross-linking agent time
PVA: Polyvinyl alcohol, Na-Alg: Sodium alginate

Table 3. Water content of PVA: Na-Alg microspheres

PVA: Na-Alg	Purified water	pH: 1.2	pH 6.8	pH 7.4
1:1 (O)	**70.1% ± 5.6**	**65.1% ± 3.2**	**105.1% ± 3.4**	**109.8% ± 7.9**
2:2 (P)	75.6% ± 6.6	88.1% ± 7.6	108.1% ± 6.3	108.7% ± 4.5
3:1 (K)	80.3% ± 4.8	90.1% ± 5.2	110.7% ± 3.8	115.7% ± 6.8

PVA: Polyvinyl alcohol, Na-Alg: Sodium alginate

Repeatability

Repeatability results are shown in Table 7. O5 results are close to each other, and the SD value is quite low. It can be concluded that the proposed system provides repeatable results.

Characterization of the microspheres

Fourier transform infrared spectroscopy (FT-IR) results

In Figure 7, the FTIR results for PVA, Na-Alg, and the blank microsphere (O) are presented. In Figure 8, the FTIR results for fampridine, the blank microsphere (O), and the fampridine-loaded O5 microsphere are shown. The broad band observed at 3377 cm^{-1} of PVA is believed to be due to the O-H stretching vibration band. The band observed around 2921 cm^{-1} is thought to be the aliphatic C-H stretching band. In the Na-Alg FTIR spectrum, the broad band around 3428 cm^{-1} corresponds to the O-H stretching band, the band at 2928 cm^{-1} corresponds to the aliphatic C-H band, and the band at 1618 cm^{-1} corresponds to the (>C = O) group.

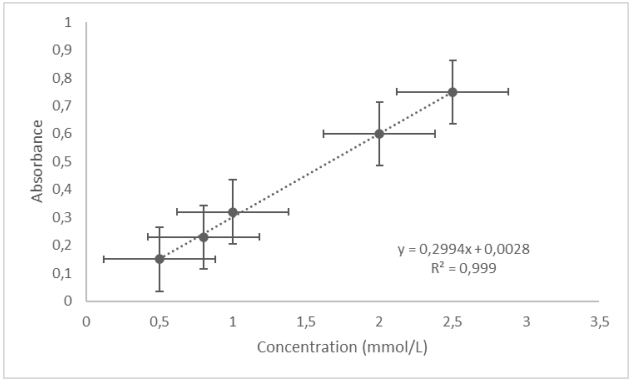

Figure 6. Linearity and range graph of fampridine.

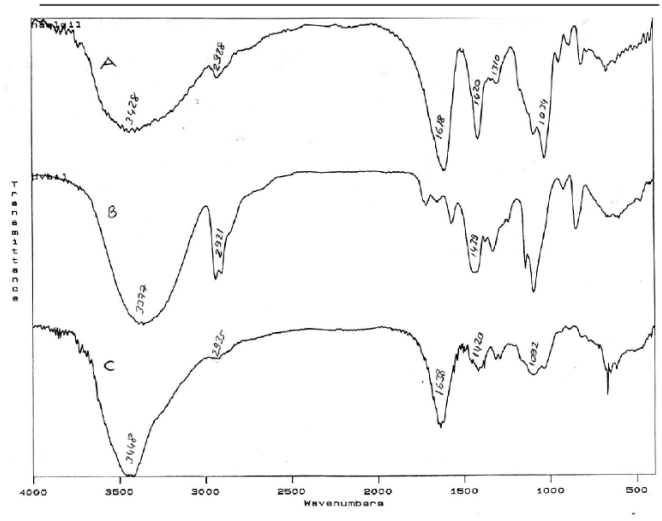

Figure 7. FT-IR results PVA (A), Na-Alg (B), and empty microsphere O (C)

Table 4. EE and particle size of microspheres

	PVA: Na-Alg	d:p	EE%	Mean particle size (μm)*
O1	1:1	1:0.5	73.5 ± 6.6	640.6 ± 1.2
O2	1:1	1:1	72.1 ± 2.2	535.3 ± 0.9
O3	1:1	1:1.25	64.7 ± 1.3	485.4 ± 1.1
O4	1:1	1:1.5	60.1 ± 5.6	360.8 ± 0.6
O5	1:1	1:2	40.4 ± 0.1	310.4 ± 0.7
P1	2:1	1:0.5	79.1 ± 3.8	710.3 ± 1.6
P2	2:1	1:1	74.5 ± 6.1	595.6 ± 1.3
P3	2:1	1:1.25	72.1 ± 4.3	520.2 ± 0.9
P4	2:1	1:1.5	60.6 ± 2.4	390.3 ± 0.6
P5	2:1	1:2	45.8 ± 1.8	340.7 ± 0.7
K1	3:1	1:0.5	87.4 ± 3.2	780.6 ± 2.1
K2	3:1	1:1	80.8 ± 3.9	630.4 ± 1.8
K3	3:1	1:1.25	75.1 ± 4.8	580.4 ± 1.4
K4	3:1	1:1.5	61.3 ± 2.9	406.8 ± 1.3
K5	3:1	1:2	55.7 ± 3.1	380.3 ± 1.5
L1	3:1	1:1.25	50.4 ± 6.2	532.3 ± 0.9
L2	3:1	1:1.25	80.1 ± 3.6	714.2 ± 0.6
M1	3:1	1:1.25	80.8 ± 2.8	695.6 ± 0.4
M2	3:1	1:1.25	54.6 ± 4.2	510.8 ± 0.2

*± SD, n= 3. d:p: Drug: polymer, PVA: Polyvinyl alcohol, Na-Alg: Sodium alginate, EE: Encapsulation efficiency, SD: Standard deviation

It was observed that in the PVA: Na-Alg microspheres, the stretching band of (>C = O) shifted to 1638 cm^{-1} due to its incorporation into the blend structure, and the aliphatic C-H band in the empty microsphere shifted from 2935 cm^{-1} to 2928 cm^{-1} upon incorporation of the drug into the structure.

Table 5. Linearity and range results

Linearity level %	Fampridine concentration (mmol/L)	Absorbance
50%	0.5	0.15
80%	0.8	0.23
100%	1.0	0.32
200%	2.0	0.60
250%	2.5	0.75
Average		0.41
Standard deviation		0.25

Table 6. System precision results

Sample (1 mmol/L)	Absorbance
1	0.32
2	0.30
3	0.28
4	0.31
5	0.29
6	0.32
Average	0.30
SD	0.016
RSD%	5.3
Confidence range (n= 6)	0.30 ± 0.01

Table 7. Repeatability results

	1st day	2nd day
Sample (O5)	Absorbance	Absorbance
1	0.27	0.26
2	0.28	0.28
3	0.29	0.28
4	0.27	0.27
5	0.28	0.29
6	0.28	0.28
Average	0.28	0.28
SD	0.007	0.01
RSD%	2.7	3.7
Confidence range (n= 6)	0.28 ± 0.006	0.28 ± 0.008

SD: Standard deviation, RSD: Relative standard deviation

DSC results

DSC diagrams of PVA, Na-Alg, and PVA: Na-Alg 1:1 microspheres (O5) are shown in Figure 9. Results showed that the Tg value of PVA was 87 ˚C, the PVA: Na-Alg microsphere is 95 ˚C, and the Na-Alg is 102 ˚C. The higher Tg value of the PVA: Na-Alg microspheres compared to PVA and lower Tg value compared to Na-Alg indicates the compatibility of these polymers.[35]

SEM results

SEM analysis was performed with the highest release observed in the O5 and empty microspheres (O). Results are shown in Figure 10. It was observed that after loading the drug into the microspheres, the surface became roughened, and there was a tendency toward shape distortion.

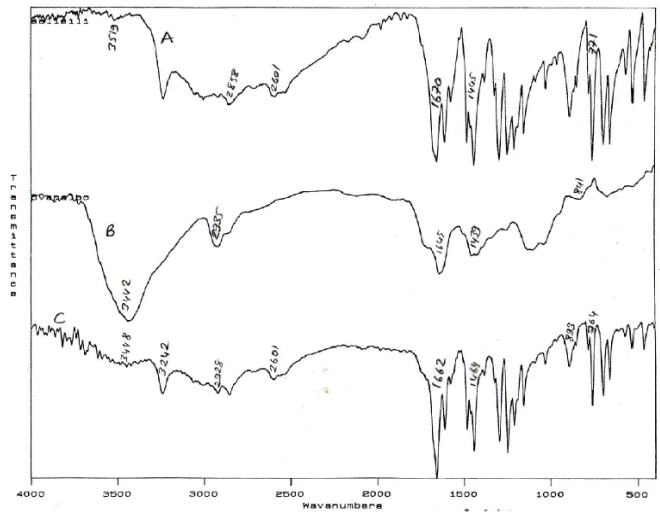

Figure 8. Fampridine (A), empty microsphere O (B), and microsphere O5 (C)

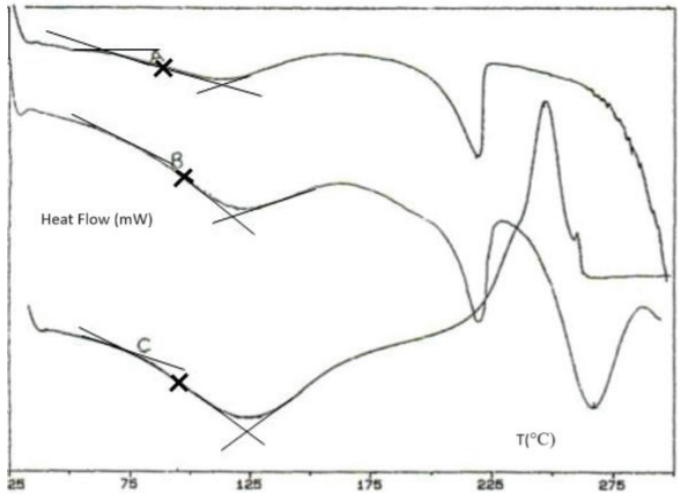

Figure 9. DSC results. PVA (A) O5 microspheres, (B) PVA: Na-Alg (C)

DSC: Differential scanning calorimetry, PVA: Polyvinyl alcohol, Na-Alg: Sodium alginate

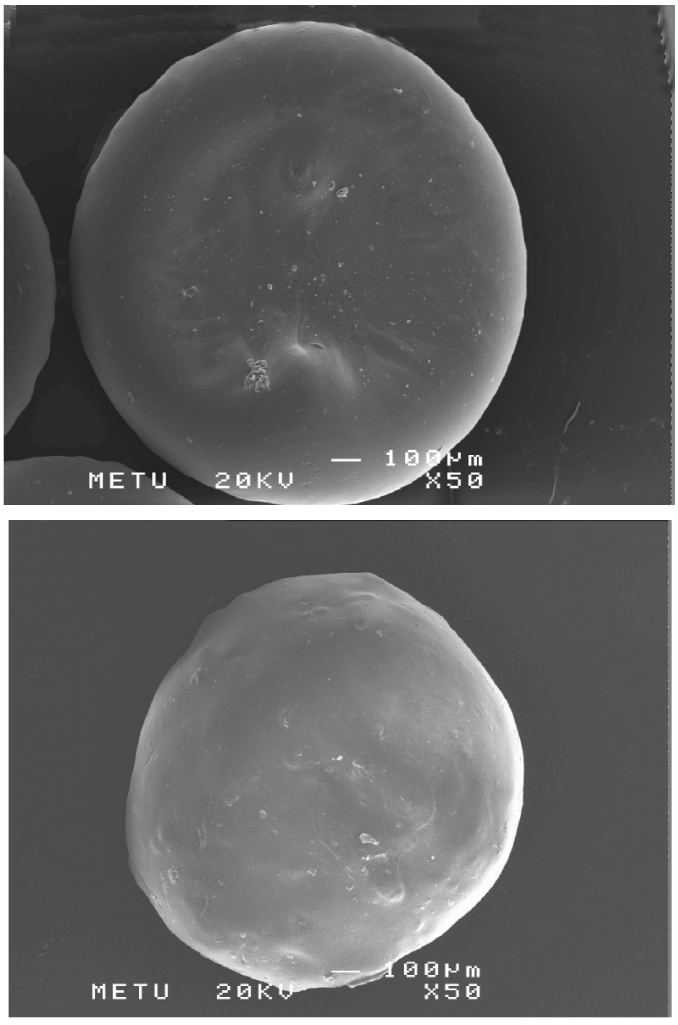

Figure 10. SEM results of O and O5 microspheres
SEM: Scanning electron microscopy

CONCLUSION

It was observed that as the amount of PVA increased in PVA: Na-Alg microspheres, the release decreased and determined that drug release in the microspheres generally increased as the d:p ratio decreased. The optimal d:p *(w:w)* ratio for PVA: Na-Alg microspheres was found to be 1:2. It was observed that an increase in the concentration of the cross-linking agent and the cross-linking time resulted in a decrease in the release rate. It was determined that drug release from the microspheres was affected by the pH of the environment. As the pH value increased, the release of fampridine from PVA: Na-Alg microspheres also increased, with the best release rate observed at pH 7.4. As a result of the studies, it was determined that fampridine release was highest (89%) in microspheres (O5) prepared with a PVA: Na-Alg *(w: w)* ratio of 1:1, and d:p ratio of 1:2 *(w:w)*, in a cross-linking solution with a concentration of 2.5% GA + 3.0% HCl *(v:v)*, and with a cross-linking time of 5 minutes.

DISCUSSION

The results of this study underscore the effectiveness of using bio-based microspheres for the controlled release of fampridine. The optimal formulation, achieved with a PVA:Na-Alg ratio of 1:1, a drug-to-polymer ratio of 1:2, and specific cross-linking conditions, demonstrated the highest release rate of fampridine. These findings are consistent with previous research indicating that the balance between polymer types, cross-linking agents, and ratios significantly influences drug release kinetics. The use of PVA and sodium alginate as biocompatible polymers is particularly advantageous as they allow for a controlled and sustained release, which is crucial for the management of conditions like multiple sclerosis, where consistent therapeutic levels of the drug are desired. The observed increase in particle size with a higher d:p ratio suggests that the drug is more effectively encapsulated in larger microspheres, which may provide enhanced stability and release control. While the results are promising, further studies are needed to explore the long-term stability of these microspheres, their *in vivo* behavior, and their comparability to existing treatments, such as Fampyra 10 mg, in clinical settings.

Ethics

Ethics Committee Approval: There is no requirement for ethical approval.

Informed Consent: Not required.

Authorship Contributions

Concept: S.A.Ö., F.Y.G., Design: S.A.Ö., F.Y.G., Data Collection or Processing: S.A.Ö., F.Y.G., Analysis or Interpretation: S.A.Ö., Literature Search: S.A.Ö., Writing: S.A.Ö.

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