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Research Article

Effect of temperature and light on the release and stability of nanoencapsulated folic acid

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Abstract

Folic acid (FA) is an essential component i.e. Vitamin B9 required at all age groups not synthesized by human body, and hence must be obtained through the dietary sources. Being vitamin it is water soluble in nature and its deficiency may lead to various disorders and diseases. Its bioavailability further decreases when exposed to temperature and light. Nanoencapsulation offers protection to the bioactive compound from adverse environmental conditions and improves the bioavailability, stability and controlled release at targeted site. In the present study the electrospraying technique has been used to nanoencapsulate the folic acid. The nanoencapsulated folic acid was exposed to temperatures (4°C and 37°C) and light (natural and UV light). After exposure to 37°C for 240 minutes, the maximum release of the nanoencapsulated folic acid of 75.11 µg, was noted. When exposed to UV light, the amount of free folic acid decreased the most, by 33.10 µg, compared to 22.73 µg when exposed to natural light.

Keywords: Folic acid, Nanoencapsulation, Electrospraying, Whey protein concentrate

Highlights

- The highest release of the nanoencapsulated folic acid, i.e. 75.11 µg was observed at 37°C up to 240 minutes
- Highest reduction was found in the free folic acid when exposed to UV light, i.e. 33.10 µg as compare to natural light i.e. 22.73 µg

INTRODUCTION

Folic acid is one of the bioactive components which can be explored for the nutraceutical application. It takes participation in several functions like biosynthesis of amino acids, nucleotides, neurotransmitters, and certain vitamins. So, it is required at all age health group (Sijilmassi, 2019).The deficiency of folic acid can cause various health issues such as anaemia, neural tube defect during the pregnancy, heart disease, cancer, dementia and cognitive functions etc. (Greenberg et al., 2011).

Gonmei and Toteja (2018) reviewed the data of micronutrient status of Indian population and reported prevalence of folate deficiency in different population of people in selected states of India ranged from 1.5 % to 63.2%. In a systematic review by Allagh et al., (2015), reported high incidences of neural tube defects (NTDs) at birth time in India i.e. around 4.5 per 1000 births, compared to other regions of the world.

The bioavailability of food folates is influenced by several parameters like the intestinal environment, chemical and physical stability/instability of folates, the composition of food and food matrix and other factors (McNulty & Pentieva, 2004; Crider et al., 2022; McNulty, 2022). The bioaccesibility/ bioavailability of folate varies from 38% to 98% from the natural sources (Brouwer et al., 1999; Brouwer et al., 2001; McNulty & Pentieva, 2004; Winkels et al., 2007; Moretti et al., 2014).

Natural form of the bioactive components like vitamins, essential oils, peptides etc. are very sensitive to the external conditions like oxygen, light, heat, pH etc. and they are susceptible to oxidation or degradation in gastro intestinal conditions. In the food system, these natural bioactive components are poorly stable, very less soluble in water and is having very poor bioavailability. Application of nanoencapsulation techniques for various bioactive components is increasing nowadays because it protects the component of choice in the gastrointestinal environment and increase the stability, solubility, absorption in the body, functionality as well as bioavailability because of smaller size and higher surface area (Chen et al., 2006; Neethirajan & Jayas, 2011; Anandharamakrishnan, 2013; Ezhilarasi et al., 2013; Bazana et al., 2019; Pateiro et al., 2021).

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Fathi et al., (2018), in their review they mentioned about the physicochemical properties of certain protein which are used for the delivery of food bioactives.

Electrospraying technique for nanoencapsulation is basically the electrohydrodynamic processing methodology and these techniques are newer and not used vastly (Pérez-Masiá et al., 2014; Raval & Ramani, 2019). These techniques are proving to be excellent choices for the nanoencapsulation in the food industry (Pérez-Masiá et al., 2014; Ghorani & Tucker, 2015). The electrospraying setup is divided in to main four parts i.e. a high voltage source of 1-30 kV, the needle or capillary to hold the suspension, syringe pump to regulate the pressure or flow rate and a grounded collector plate or rotating drum (Kessick et al., 2004; Raval et al., 2019). In this electrospraying process, the evaporation of the solvent results in to solidification and narrowing of the droplets and finally the solid polymeric particles deposited on the collector (Nguyen et al., 2016).

The present investigation explored the folic acid nanoencapsulation through electrospraying and exposed it to temperature of 4°C and 37°C to study the release pattern of nanoencapsulated folic acid as well as it is also exposed to natural light and UV light to check its stability.

MATERIALS AND METHODS

Preparation of solution and optimization study of folic acid-whey protein concentrate (WPC)-proline-lactate for electrospraying: The folic acid (Sigma Aldrich) solution was prepared as per the manufacturer's instruction *i.e.* in 1 M NaOH (40 mg/mL) (SD Fine chemicals) in amber coloured bottle. And the WPC (AS-IT-IS brand) solution was prepared in different concentration i.e. 10%, 20%, 30% and 40%. Various combination ratios of proline (P) (Sigma Aldrich) and lactate (LA) (Sigma Aldrich) with WPC and folic acid were used to prepare solutions for nanoencapsulation through electrospraying. The electrospraying was carried out using Fluidnatek LE-10 (Bioincia S. L., Valencia, Spain), equipped with a variable high voltage power supply. The prepared solutions were subjected to electrospraying at different flow rates, voltage and tip-to collector distances.

Standard curve preparation for the quantification of folic acid: The quantitative analysis of folic acid was carried out by using the method described by Kshirsagar et al. (2017) with slight modification. The absorbance was measured at λ_{max} (281 nm) in double beam spectrophotometer (Model - UV- 1900, Make - Shimadzu, Japan). The procedure for standard curve preparation was adapted from Olmo et al. (2022) with slight modification. Different concentrations of folic acid in the range of 100 µg/mL to 500 µg/mL were prepared in 1 M NaOH solution. Measurement for the calibration was done using the 1 M NaOH solution as a blank. The stock solution was prepared by dissolving 100 mg folic acid and in a 100 mL volumetric flask in 1M NaOH. The working standard solution of 100 µg/mL was prepared by diluting the 0.1 mL of stock solution in 10 mL of distilled water.

Study of release profile at different temperature: The release behavior of nanoencapsulated folic acid at 4°C and 37°C in refrigerator and incubator (pre-controlled), respectively was studied by drawing samples at an interval of 0, 30, 60, 120, 150, 180, 210 and 240 minutes. The concentration of released folic acid was measured using a UV-VIS spectrophotometer.

Stability study of nanoencapsulated folic acid against photodegradation: The method described by Vorobei and Vorobei (2011) was adopted with minor modification to study the photodegradation of nanoencapsulated folic acid. The photo-stability of nanoencapsulated folic acid was evaluated by exposing samples to natural light and to UV light in a UV chamber (Make- LTC, India) (λ_{max} - 365 nm, power density - 12 W/m^2 was used and the range was 315-400 nm with supply voltage is 230v/50 Hz.). Both nanoencapsulated folic acid and free folic acid were exposed to the natural light for up to 30 days and UV light continuously for 4 hours.

Under natural light exposer the concentration of folic acid was measured on 0, 5, 10, 15, 20, 25 and 30 days while under the UV light. The concentration of folic acid was measured at 0, 30, 60, 120, 150, 180, 210 and 240 minutes using UV-VIS spectrophotometer.

Statistical analysis

The collected data were subjected to statistical analysis by analysis of variance (ANOVA). All analyses were performed with 3 replications the different treatments means were compared by critical difference test at 5% level of significance ($p \leq 0.05$) with the help of WASP (Web Agri Stat Package) developed by the Indian Council of Agricultural Research (ICAR), New Delhi (https://ccari.icar.gov.in/wasp/index.php).

Table 1. Optimized process of electrospraying for nanoencapsulation of folic acid

Folic acid	WPC	P:LA ratio	Flow rate (µL/hr)	Voltage (kv)	Distance (cm)
150 µg	10%	1:1	300	15	13

RESULTS

Preparation of solution and optimization study of folic acid-WPC-proline-lactate for electrospraying: The optimized formulation for the nanoencapsulation of folic acid through electrospraying process consisted of 150 µg folic acid, 10% WPC and proline to lactate ratio of 1:1. The optimized parameters including flow rate, voltage and a tip-to-collector distance in the electrospraying process is mentioned in Table 1. The encapsulated powder was collected on flat collector was wrapped with parchment paper in aluminium foil and packed in sterile sealed polythene bags and stored at 4°C and 37°C for further analysis. The obtained powder samples were subjected for further analysis.

Preparation of standard curve for release profile and stability study: The absorbance values of folic acid is shown in Table 2 and Fig. 1. A standard curve of folic acid was prepared by using different concentration of water soluble folic acid in 1 M NaOH solution. The optimum wavelength for maximum absorption of water soluble folic acid (λmax) is 281 nm (Kshirsagar et al., 2017). The absorbance of standard folic acid solution was measured at 281 nm against distilled water as a blank. Standard curve was plotted with absorbance against concentration.

Table 2. Absorbance of folic acid at its different concentration in distilled water

No.	Concentration of folic acid (µg/mL)	Absorbance (WL- 281)
1	2	0.11
2	4	0.21
3	6	0.32
4	8	0.43
5	10	0.53

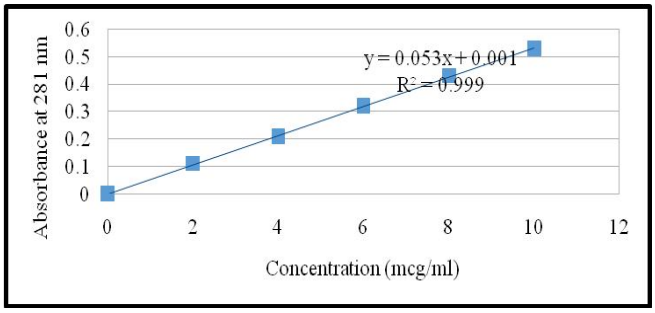

Fig. 1. Standard curve of folic acid

Release profile of nanoencapsulated folic acid at different temperature: The developed nanoencapsulated folic acid was intended to fortify the yoghurt, which is generally stored at 4 °C and after consumption it is exposed to the body temperature (37°C). Therefore, the objective of this study was to check the release of nanoencapsulated folic acid at 4°C and 37°C storage temperature.

The concentration of nanoencapsulated folic acid released at 4°C and 37°C is shown in Table 3. The maximum concentration of released folic acid was found to be 75.14 µg at 37°C after 240 minutes of exposure. At 4°C, the maximum concentration of nanoencapsulated folic acid reached was 70.21 µg after 210 minutes of exposure and followed by reduction in the folic acid.

Table 3. Concentration of nanoencapsulated folic acid released at 4°C and 37°C

	Concentration (µg) of folic acid	
Minutes	**4 °C**	**37 °C**
0	56.47^{h}	56.69^{h}
30	56.76^{g}	57.29^{g}
60	58.04^{f}	58.47^{f}
120	58.99^{e}	60.00^{e}
150	60.07^{d}	61.30^{d}
180	61.05^{c}	66.04^{c}
210	67.62^{a}	69.53^{b}
240	65.62^{b}	75.11^{a}

The percentage release of nanoencapsulated folic acid is given in Fig. 2. At 37°C storage temperature, the maximum release of nanoencapsulated folic acid was 50.08% up to 240 minutes. In contrast, at 4°C storage temperature, the maximum release was 45.08% after 210 minutes, followed by reduction in the concentration of the nanoencapsulated folic acid to 43.74% at 240 minutes.

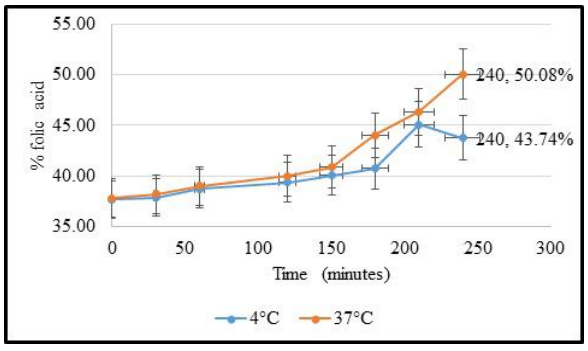

Fig. 2. Percentage release of folic acid at different time interval and temperature

Photodegradation of nanoencapsulated folic acid under natural light and ultraviolet (UV) radiation: The objective of this study was to check the photodegradation of nanoencapsulated folic acid for its application in the food. To check the stability of nanoencapsulated folic acid (NEFA) and free folic acid (FFA) against the photodegradation, NEFA and FFA were exposed under natural light and UV irradiation. The concentrations of NEFA and FFA was quantified at 0, 5, 10, 15, 20, 25 and 30 days under natural light exposure. The concentration of FFA and NEFA was quantified at 0, 30, 60, 120, 150, 180, 210 and 240 minutes under UV light exposure. Initial concentration of folic acid was 37.87 µg.

The concentrations of FFA and NEFA after natural light exposure is given in Table 4. During 30 days exposure of natural light, there was less degradation of folic acid in NEFA as compare to FFA. The concentration of folic acid in nanoencapsulated folic acid, was reduced up to 27.63 µg i.e. 27.04% whereas the free folic acid was reduced up to 16.24 µg i.e. 57.11% after 30 days of storage.

Table 4. Concentrations of FFA and NEFA under exposure of natural light

Days	Concentration (µg)	
	Free Folic Acid	Nanoencapsulated Folic Acid
0	37.87[a]	37.87[a]
5	28.56[b]	29.57[b]
10	25.57[c]	29.31[c]
15	23.32[d]	28.89[d]
20	22.46[e]	28.03[e]
25	19.53[f]	27.98[f]
30	16.24[g]	27.63[g]

The concentrations of free folic acid and nanoencapsulated folic acid after natural light exposure are given in Table 4. Under UV light exposure for up to 240 minutes, there was less degradation of folic acid in nanoencapsulated folic acid as compared to free form of folic acid. The concentration of folic acid was decreased to 33.10 µg i.e. 12.60% in nanoencapsulated folic acid, whereas the free folic acid was declined to 22.73 µg i.e. 39.99% up to 240 minutes.

Table 5. Concentrations of FFA and NEFA under exposure of UV exposure

Exposure time(Minutes)	Concentration (µg)	
	Free folic Acid	Nanoencapsulated folic acid
0	37.87[a]	37.87[a]
30	31.33[b]	37.11[b]
60	30.75[c]	36.89[c]
120	29.54[d]	36.25[d]
150	27.56[e]	35.79[e]
180	25.65[f]	35.01[f]
210	23.15[g]	34.27[g]
240	22.73[h]	33.10[h]

The percentage reduction in FFA and NEFA under natural light is given in Fig. 3. When exposed to natural light for 30 days photodegradation of NEFA was, 27.04%. The degradation increased gradually by 21.92% at 5 days, 22.60% at 10 days, 23.71% at 15 days, 25.98% at 20 days and 26.12% at 25 days, respectively. In contrast, the degradation of FFA increased to 24.58% on 5th day, 32.47% on 10th day, 38.42% on 15th day, 40.70% on 20th day and 48.43% on 20th day of natural light exposure. FFA shown 57.11% of degradation after the exposure after 30 days.

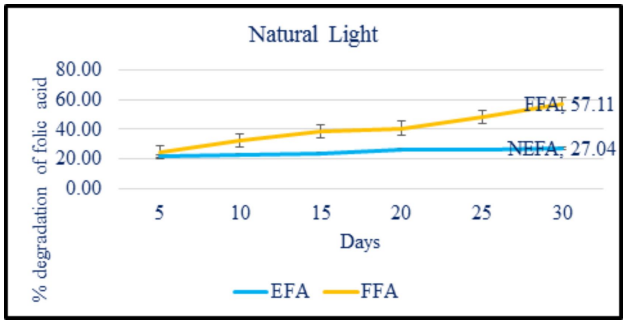

Fig. 3. Percentage of folic acid degradation in natural light

The percentage of reduction in FFA and NEFA under UV exposure is given in Fig. 4. After 240 minutes exposure to UV irradiation NEFA showed 12.60% of degradation. The degradation was increase gradually with time showed 2.01% at 30 minutes, 2.59% at 60 minutes, 4.28% at 120 minutes, 5.49% at 150 minutes, 7.55% at 180 minutes and 9.51% at 210 minutes of UV exposure. In case of FFA, there was 39.99% of degradation was found under UV irradiation after the exposure up to 240 minutes. The degradation

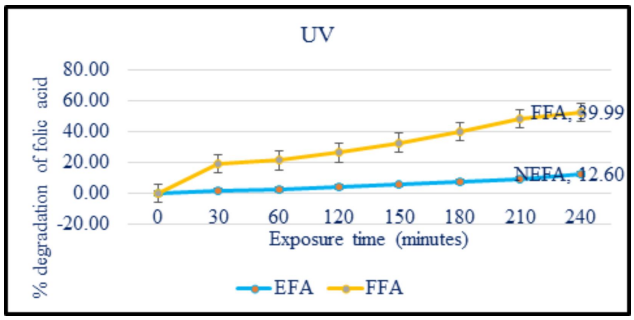

Fig. 4. Percentage of folic acid degradation under UV exposure

was followed by 17.28% at 30 minutes, 18.79% at 60 minutes, 21.99% at 120 minutes, 27.23% at 150 minutes, 32.26% at 180 minutes and 38.86% at 210 minutes of UV exposure.

Percentage of photodegradation was higher in free folic acid under natural light as well as UV light. Whereas, there was lower photodegradation in nanoencapsulated folic acid as compare to free folic acid.

DISCUSSION

Release profile of nanoencapsulated folic acid at different temperatures: Six to eight hours are normally required for the food to pass through stomach and small intestine after which it enters large intestine where remaining digestion and absorption of water takes place. The movement of food through the entire colon is takes about 36 hours (Leonard, 2023).

In presented study the release of NEFA was increased gradually with time at 37°C, which suggest higher probability of higher absorption in the intestine. This release behaviour result of folic acid accords with previous scientific literatures.

Mohammed et al., (2021) used *Lycopodium clavatum*s poropollenin (LCS) as a coating material for the encapsulation of folic acid and reported that the average percentage of cumulative release of encapsulated folic acid was 76.1% in pH 7.4 and 45.5% in pH 1.2 when stored at 37±0.5°C for 10 hr.

Similarly Britto et al., (2016) prepared the nanocapsules of vitamin C, folic acid and vitamin B12 through ionic gelation process of chitosan and tripolyphosphate and reported retention of 72% of folic acid after 15 days of storage at ~ 25 °C. Moholkar et al., (2021) encapsulated the folic acid with phospholipids of peanut seeds through liposomal method and observed approximately 43% release of encapsulated folic acid up to 4 hours at 37°C.

Prasertmanakit et al. (2009) prepared microcapsules of folic acid using ethyl cellulose and span 80 by oil-in-oil emulsion method and reported approximately 45% release of encapsulated folic acid with 2% span 80 up to 4 hours at 37°C.

Prosapio et al. (2015) prepared the complex of polyvinylpyrrolidone and folic acid using supercritical antisolvent precipitation method and reported approximately 40% release of folic acid at 37°C up to five hours. Song et al., (2017) reported the ≈ 38% release from folic acid nanofibers at 37°C up to 250 minutes. Èrnivec et al., (2020) also reported the higher levels of folic acid release rates at elevated temperatures.

Our results are at par with above studies. At 37°C the release of NEFA was found higher i.e. 75.11 µg and the percentage of free folic acid was gradually increased as compared to 4°C. The temperature of human body is also about 37°C, thus after ingestion of nanoencapsulated folic acid, maximum folic acid will be available for the absorption in the intestine.

Photodegradation of nanoencapsulated folic acid under natural light and ultraviolet (UV) radiation: The folic acid is sensitive to UV light (Araújo et al., 2011; Borradale et al., 2014; Csapó et al., 2019), which is due to excitation of bond between C9 and N10 of folic acid which results in the formation of 6-formylpterin and p-aminobenzoyl- L-glutamic acid, as photodegradation products (Juzeniene et al., 2013).

Fu et al. (2018) studied the photodecomposition of free folic acid and whey protein-folic acid complex and found photodecomposition of free folic acid was 50 µM to 7 µM within 40 minutes of irradiation whereas, in whey protein-folic acid complex for same level degradation took more than 90 minutes. The authors concluded that the whey protein serves as a good carrier material for folic acid encapsulation. In this study, we used WPC for the nanoencapsulation of folic acid suggest that the encapsulated folic acid could be protected by the WPC.

Madziva et al. (2006) evaluated the retention of free folic acid in the cheddar cheese and found degradation of 58.33% of free folic acid after month. Chapeau et al., (2017) encapsulated the folic acid-whey protein through the coacervation method. They used UV light of 254 nm radiation energy for the photodegradation study of free folic acid and encapsulated folic acid. They reported the H ≈ 35% degradation of free folic acid after the 5 hr exposure.

Attaf and Hasan (2019) prepared the folic acid solution of different concentration i.e. 10, 20, 30, 40 and 50 ppm and it was exposed to UV for the time interval of 5, 10, 15, 20 and 25 minutes in the UV cabinet and checked their concentration through spectroscopic method indifferent solvents such as water, DMSO, acetic acid etc. They found the degradation of folic acid after 15 minutes of exposure and the concentration was around 36 ppm and after 25 minutes of exposure it was decreased to 31 ppm.

Juzeniene et al. (2013) studied the photodegradation of folic acid (100 mM) in aqueous solution and they used 260-400 nm wavelength. They reported that the rate of photo-degradation of folic acid was dependent on wavelength and not on the concentration. They also mentioned that the degradation was higher in UBA than UVB region but

overall the photo-oxidation was not more than 5% of folic acid.

Pérez-Masiá et al. (2015), encapsulated the folic acid using WPC and observed 23.51% degradation of encapsulated folic acid after 15 days of exposure in natural light which was further degraded to 27.64% after 30 days. In the study of doEvangelho et al., (2019), the encapsulated folic acid was exposed to UVA radiation up to 24 hours and reported, 12.33% degradation of encapsulated folic acid after 1 hour of UV exposure. The results of this study showed better stability compared to above studies.

From the above findings, it may be concluded that, folic acid (150 µg), WPC (10%) and proline:lactate (1:1) ratio under optimsed conditions of electrospraying can be used to prepare NEFA. The nanoencapsulated folic acid is more stable as compare to free folic acid when exposed to natural light as well as UV light. Furthermore, the maximum release of nanoencapsulated folic acid was found at 37°C indicated that it may effectively release in the human body after consumption. This nanoencapsulated folic acid prepared is suitable for food fortification such as yoghurt and can provide improved bioavailability of folic acid and solar radiation.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: MH: Involved in investigation, data generation, preparing original draft; VM: Engaged in conceptualization, data curation, supervision and final editing; KG, TH: Involved in statistical analyses, methodology and editing.

Data availability statement: Research data regarding replication and statistical analysis was conducted and authors have data related to results published in this research paper and will be made available on demand.

Ethical statement: Work done in research paper was not involved any animal studies, hence there is no need for approval of the ethical committee.

Use of artificial intelligence tools: Authors have not used any artificial intelligence tools in this study. The author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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