

Manufacture and Assessment of the absorption capability of famotidine to 3D-nano-cellulose network

Van Hung Mai¹, Xuan Thanh Nguyen², Phuong Lien Lai³, Thuy Linh Do³, Thi Phuong Le^{3*}

¹Research Center for Anthropology and Mind Development, VNU University of Education, Vietnam

²Hanoi Pedagogical University 2, Vietnam

³VNU University of Education, Vietnam

*Corresponding author: lethiphuong@vnu.edu.vn

ABSTRACT

Background: Famotidine is one of gastrointestinal drugs which is used by drink or injection, soluble in acids and weakly dissolved in water. It has the effect of reducing gastric secretion by inhibiting histamine at the H₂ receptor in the gastric mucosa wall, decreasing the secretion of HCl in gastric juice, the healing of gastric ulcers. Manufacture, *In vitro* Characterization And *In vivo* Bioavailability Evaluation of famotidine Loaded 3D-Nano-Cellulose Network (3DNC) by bacteria and Used for Oral Administration.

Methods: Famotidine is a gastrointestinal H₂-receptor antagonist drug with low bioavailability (40-45%). 3DNC can be produced from bacteria living in fermented green tea and is the material containing nano-sized fibers which is capable of loading Famotidine to form a prolonged release therapy to improve drug bioavailability.

Results: This research has been produced 3DNC materials from bacteria in some nutrient media; it has used scanning electron microscope (SEM) to determine the 3DNC structure, spectrophotometer FT-IR to determine drug interaction to 3DNC, high performance liquid chromatography (HPLC) to determine Famotidine in rabbit plasma.

Conclusion: Three-dimensional nano-cellulose network has an involvement of the nano-sized cellulose fibers with three dimensional networks that are capable of loading Famotidine drug.

Keywords: Bioavailability, Famotidine, oral delivery, Bacteria

Correspondence:

Thi Phuong Le

³VNU University of Education, Vietnam

*Corresponding author: lethiphuong@vnu.edu.vn

INTRODUCTION

Rationale: Famotidine is one of gastrointestinal drugs which is used by drink or injection (fig 1), soluble in acids and weakly dissolved in water [1]. It has the effect of reducing gastric secretion by inhibiting histamine at the H₂ receptor in the gastric mucosa wall, decreasing the secretion of HCl in gastric juice, the healing of gastric ulcers.

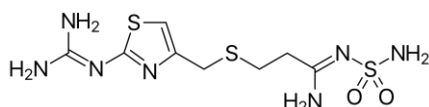


Fig 1. Structure of Famotidine

However, the low bioavailability of Famotidine (about 40-45%) has prevented its therapeutic applications [1],[2]. Drinking is one of the most favorite and traditional ways to distribute drugs, reducing costs and pressure on patients [2]. Therefore, a system designed to help the drug to absorb and has slowly increased the bioavailability of the drug.

Literature review

Many researches showed that famotidine could be successfully delivered to provide night-time relief of gastric acidity by formulating floating pulsatile drug delivery system [3]. It is demonstrated that the multiple-unit floating-bio-adhesive cooperative mini tablets enhanced the oral bioavailability of famotidine in animal [1]. The study also proved that the multiple-unit floating-bio-adhesive cooperative mini-tablets might be a promising gastro-retentive delivery system for stomach therapeutic drugs. The acid function of carboxymethyl-beta-cyclodextrin in the enhancement of the chemical stability, oral-route bioavailability and bitter taste of

famotidine were evaluated [4]. The rate of release of famotidine through the construction of liquid, solid tablets in both *in vitro* and *in vivo* were shown [5]. The pharmacokinetics and bioavailability of famotidine on some Chinese volunteers were studied [6].

Information

Green tea (*Camellia sinensis*) which grows easily and well, especially in natural conditions, is often used as a healthy drink in the locality. The fermented green tea contains bacteria producing 3DNC. The metabolites of bacteria in the fermentation contain 3DNC, which has been used as a drink called Kombucha tea. The 3DNC has the structure of super-thin nano fibers, great tensile, mechanical strength and so on. Some studies have proved that the 3DNC has the potential of being a delivery system with its properties [2], [6]. The use of 3DNC on coconut jelly (made from coconut juice thanks to the fermentation of bacteria strain *Acetobacter xylinum*) coating for Paracetamol by spraying technique [7].

Overall

The results have indicated that the 3DNC membranes are able to increase releasing time of the drug and improve the efficiency of drug use. Huang L. et al. [8],[9],[10] have tested the 3DNC membrane from the fermentation of bacteria *Gluconacetobacter xylinum* in standard medium (Hestrin-Schramm) for transporting and releasing Berberine *in vitro*.

Objective

Manufacture, *In vitro* Characterization And *In vivo* Bioavailability - Assessment of the absorption capability of Famotidine to 3DNC.

Materials and Research methods

Materials and chemicals: Famotidine 99.5% (Sigma – USA); tablets Famotidine (FAMSYN-20, Haryana – India); Dialysis bag (MWCO: 12000-14000; Serva – Germany); Yeast extract (USA); Peptone (European Union); Methanol, Acetonitrile, Sodium Acetate Trihydrate, Triethylamine, Ice Acetic Acid,... (Merck) [11]; other standard chemicals used in chromatography and analysis.

Equipment

Spectrophotograph UV-Vis 2450 (Shimadzu, Japan); Analytical scale (Sartorius, Switzerland); Magnetic Stirrer (IKA, Germany); Low speed rotator (Orbital Shakergallenkump, Anh); Shaker (Lab companion, SKF-2075, Korea); Oven, Incubator (Binder, Germany); Antiseptic cabinet (Haraeus); Antiseptic autoclave (HV-110/HIRAIAMA, Japan); Liquid chromatography apparatus (Acquity UPLC HClass, Mass spectrometry Xevo TQD, Waters, USA); Rotating equipment (Vortex ZX3, Velp Scientifica, USA); Reciprocating shaking equipment (Reciprocating Shaking 3006, GFL, Germany); Centrival solvent system

(Labconco, USA); Deep fridge (MDF 236, Sanyo, Japan) [12],[13].

Sampling technique and Sample size

healthy white rabbits, weight approximately 2.5-2.7kg, the same age (around 6 months old), laboratory standard, is supplied from The National Institute of Drug Quality Control. The rabbits have adapted to laboratory conditions at least one week and have starveling in 12 hours, supplied fully water during the preparation.

Study design

Bacteria strain *Acetobacter* from fermented green tea: Boiling 1000ml water, adding 20g green tea in 10-15 minutes, filtering to get tea fluid and filling in clean glassware, adding next 100g sugar and stirring well, letting cool. After 7-10 days in 30°C distilling tea fluid with the fermentation of sugars (containing *Acetobacter* bacteria: *Acetobacter xylinum*,...) [14], [15] and agar on the surface. Trapping process of *Acetobacter* bacteria strain from fermented green tea (Fig 2)

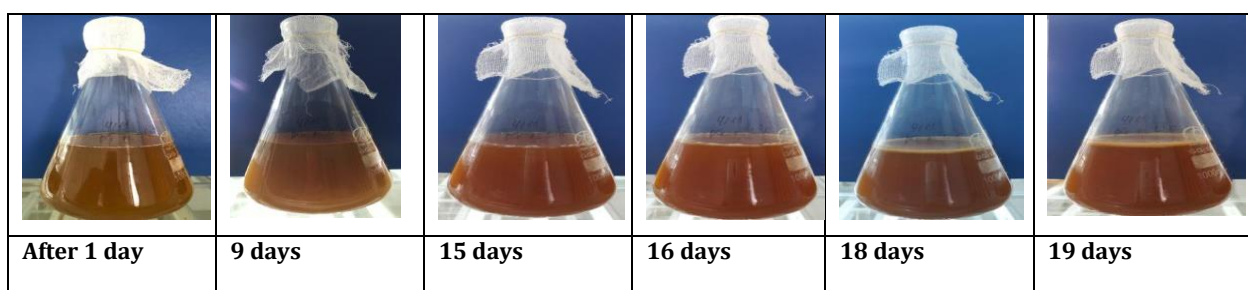


Fig 2. Trapping *Acetobacter* bacteria strain from fermented green tea

After trapping the bacteria strain from the fermented green tea, put them into the nutritional media standard medium (SM), coconut medium (CM), and rice medium (RM) to create the best environment for generating 3DNC for delivering and distributing the prolonged drug.

Inclusion criteria

Fermenting bacteria strains from three media: SM [8],[16] including glucose (20g), pentone (5g), diammonium phosphate (2.7g), yeast extract (5g), citric acid (1.15g), double-distilled water (1000ml); CM [17], [18] including glucose (20g), peptone (10g), diammonium phosphate (0.5g), ammonia sulfate (0.5g), matured coconut water (1000ml); RM [18] including glucose (20g), peptone (10g), diammonium phosphate (0.5g), ammonia sulfate (0.5g), rice water (1000ml).

Study period

from January to December 2018

Study location

Acetobacter bacteria producing cellulose from fermented aqueous green tea extract [14, 15] were cultured in the clean laboratory of Microorganism-Animal, Institute of Scientific Research and Applications (ISA)-Hanoi Pedagogical University 2 (HPU2) and Research Center for Anthropology and Mind Development, VNU University of Education.

Data analysis

All results are processed by Excel 2010 and it is performed by the mean±standard deviation (SD) and two-way

ANOVA test. The results are considered to be significant with $p < 0.05$.

Results

Manufacture of 3D-nano-cellulose network (3DNC)

Bacteria strain *Acetobacter* from fermented green tea: Boiling 1000ml water, adding 20g green tea in 10-15 minutes, filtering to get tea fluid and filling in clean glassware, adding next 100g sugar and stirring well, letting cool. After 7-10 days in conditions of 30°C distilling tea fluid with the fermentation of sugars (containing *Acetobacter* bacteria: *Acetobacter xylinum*,...) [15], [16] and agar on the surface. Trapping process of *Acetobacter* bacteria strain from fermented green.

Fermenting bacteria strains from three media:

SM [14] including glucose (20g), pentone (5g), diammonium phosphate (2.7g), yeast extract (5g), citric acid (1.15g), double-distilled water (1000ml);

CM [19] including glucose (20g), peptone (10g), diammonium phosphate (0.5g), ammonia sulfate (0.5g), matured coconut water (1000ml);

RM [2] including glucose (20g), peptone (10g), diammonium phosphate (0.5g), ammonia sulfate (0.5g), rice water (1000ml).

Assessment of the absorption capability of Famotidine to 3DNC

Perform the experiment of the famotidine (Fa) absorption into the 3DNCs which is produced from different media based on optimal conditions and drug content of the control product (tablets in the market) of 20mg. At the end of the experiment, the sample was removed from the absorbent solution to measure OD, based on the drug's

calibration curve to calculate the drug dose that was absorbed into the 3DNCs and the loading efficiencies of the 3DNCs.

The results shown in Table 1 show that there is no difference in drug intake and loading efficacy in the 3DNC types produced from different media.

Table. Drug absorbed dose into the 3DNC types

3DNC type	3DNC-SM (mean \pm SD)	3DNC-CM (mean \pm SD)	3DNC-RM (mean \pm SD)
Drug dose (mg)	20.03 \pm 0.01	20.07 \pm 0.02	20.05 \pm 0.01
Efficiency (%)	89.10 \pm 0.02	90.00 \pm 0.01	89.25 \pm 0.03

The results shown in fig 3 show that the physicochemical properties of 3DNC loaded Famotidine and the structure of 3DNC loaded drug.

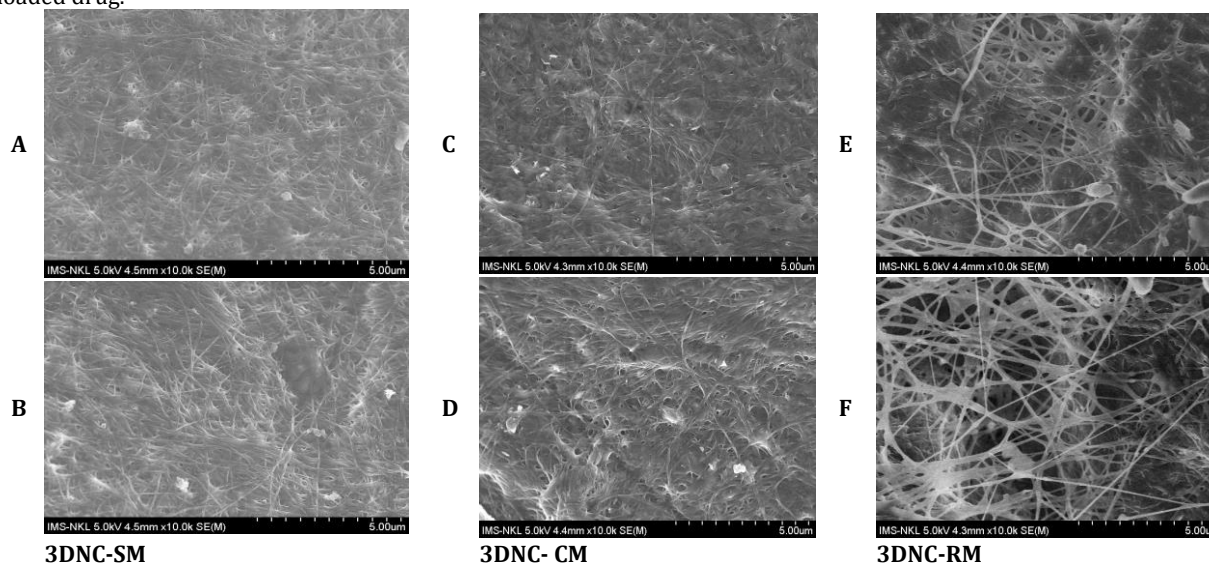


Fig 3. Pictures captured from scanning electron microscope (SEM) of 3DNC which generated from SM, CM and RM without Famotidine (A, G, E) and loaded Famotidine (B, D, F)

Determine the interaction of 3DNC to Famotidine by fourier transformation infrared (FT-IR)

Spectral measurements of the Fa, 3DNC generated from the media (SM, CM, RM) before and after loading drug are shown in Fig 4, 5, 6, 7, 8, 9 and 10.

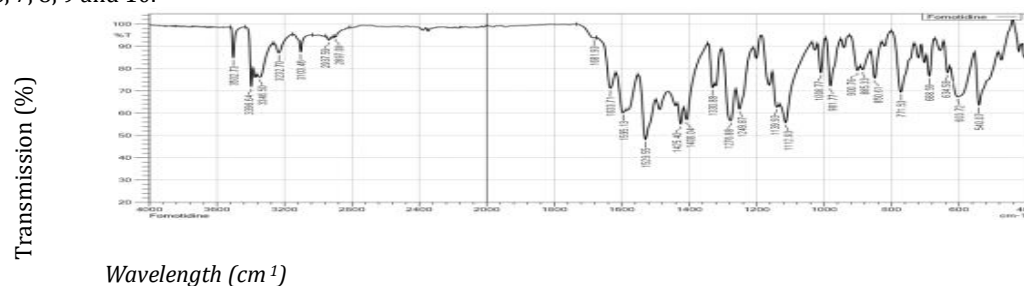


Fig 4. Spectral measurement of Famotidine

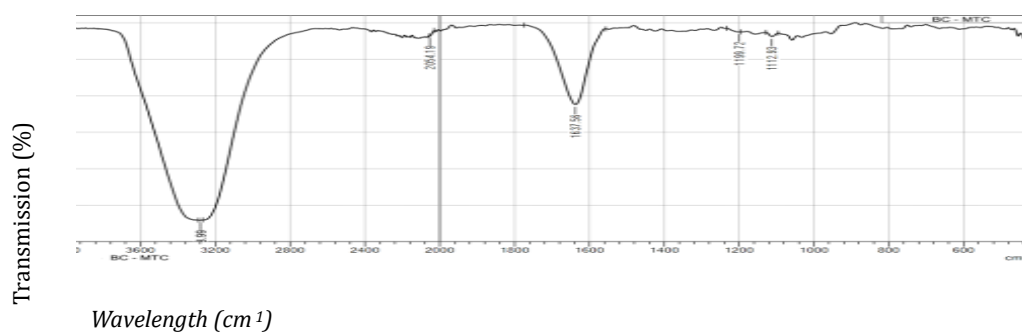


Fig 5. Spectral measurement 3DNC cultured in SM

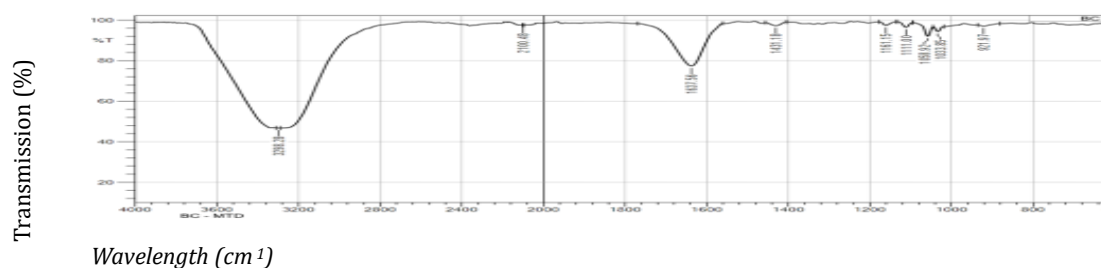


Fig 6. Spectral measurement 3DNC cultured in CM

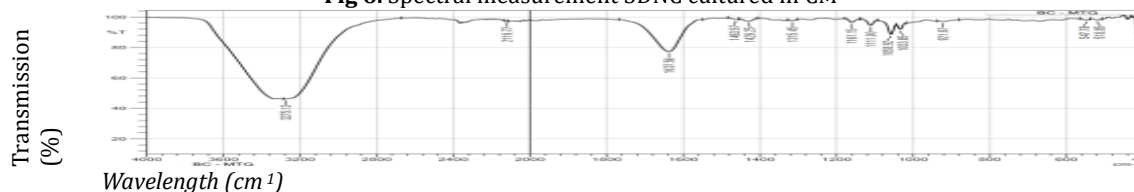


Fig 7. Spectral measurement 3DNC cultured in RM

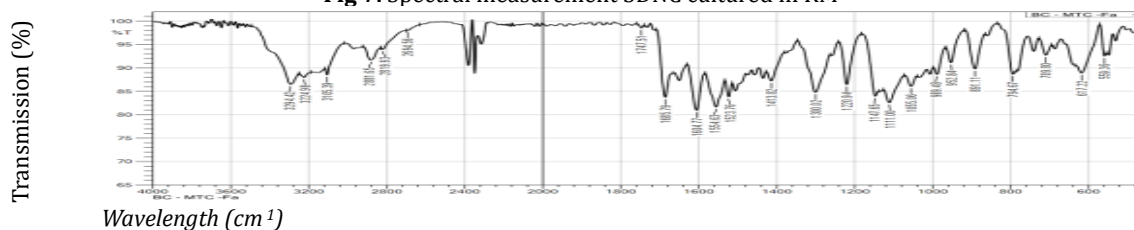


Fig 8. Spectral measurement 3DNC cultured in SM loaded Famotidine

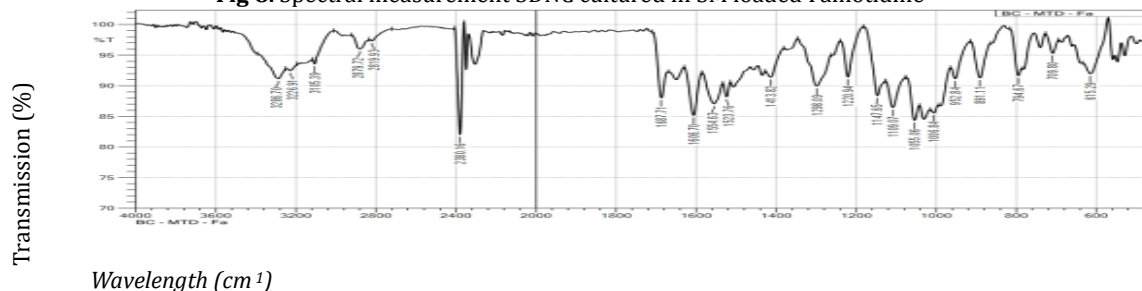


Fig 9. Spectral measurement 3DNC cultured in CM loaded Famotidine

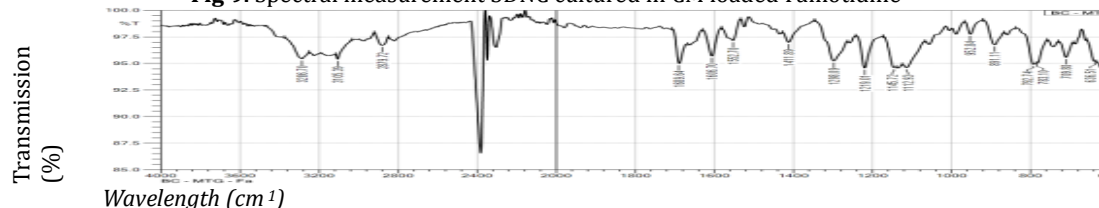


Fig 10. Spectral measurement 3DNC cultured in RM loaded Famotidine

The results of the FT-IR spectra shown in Figures 4 to 10 showed that, in the loaded 3DNC types, there is no additional peaks are associated with the formation of new complexes, but the difference in the relative magnitude of the peaks is typical for 3DNC and the drug can be observed.

Discussion

The structure of 3DNCs (cultured in SM, in CM and in RM) before and after loading drug with SEM is shown in Figure 1. As the results, the 3DNC has the homogeneous fiber structure network. These results are consistent with other studies on the structure of 3DNC including nano-sized cellulose fibers that make up the three-dimensional structure network [4], [8]. According to Huang L. et al have compared the SEM image of cultured 3DNC to SM generated from *Gluconacetobacter xylinum* after 24 hours treatment with some conditions (double-distilled water, artificial medium of stomach and intestine, NaOH medium) showed that: porosity of the 3DNC cultured in SM in acidic

and alkaline media increasing when compared to neutral medium (double-distilled water) [8]. These affirmed that have the contraction of cellulose fibers in these two conditions and neutral medium does not affect to the cellulose fibers. The results of this project show that 3DNC is loaded and non-loaded no apparent difference in results consistent with other studies [8], [13]. For the 3DNCs made of SM or CM, the cellulose fibers have the stable structure, with no significant changes in structure when loaded under optimal (experimental) conditions. For the RM type, the spatial structure of the cellulose fibers is noticeably altered after loading, the size of the holes in the membrane changes, the cellulose fibers are loosely linked, and the structure is unstable.

Compared to the BC produced from the standard culture of pure bacteria strain [8], the 3DNC structure in this project was not significantly different. The 3DNC materials and the drug have thus no covalent bonding of the loaded 3DNC material, therefore, only involving in non-covalent

bonding forces. The spectra of samples have the same properties before and after loading Fa with changes in the relative intensity of the typical peaks. As the results, the presence of the drug Fa in the structure of 3DNC. The research results of the project are also consistent with those of Huang et al. [8]. In addition, the results of the spectral analysis in the project also show that the typical peaks in the control sample are not significantly changed. Therefore, there is no modification in the chemical composition of the drug during the loading of the 3DNC type.

Conclusion

The 3DNC obtained from CM and RM has the same sizes and characteristics as the 3DNC obtained from SM and can be fabricated with the desired thicknesses and sizes in all three types of media. The manipulated 3DNC has a neutral pH and the suitable purity to guarantee the precision of the loading material.

Investigation of the 3DNC structure by SEM shows that the 3DNC cultured in SM and CM, cellulose fibers have a stable structure, without modifications in structure when loaded under optimal conditions. The FT-IR spectrophotometry confirms the interaction between Famotidine and 3DNC, the presence of Famotidine in the 3DNC structure with no change in the chemical composition of the drug during the loading of 3DNC types.

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Conflict of interests

The authors declare no conflicts of interest

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