Mucoadhesive buccal patches based on chitosan for delivery of *Vernonia cinerea*

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Abstract

Perovskite material, which exhibits ferroelectric (FE) behavior, is widely used for ferroelectric-photovoltaic (FE-PV) cell application. Generally, the bandgap of FE material is very large (E_g > 3 eV), which limits a light absorption to only the ultraviolet region of solar spectrum. Recently, the $(1-x)[KNbO_3]-x[BaNi_{0.5}Nb_{0.5}O_{3-\delta}]$ (KBNNOx) material has been discovered. This material provides very small bandgap ($E_g \sim 1.4$ eV with $x = 0.1$), but, according to the literature, has a low efficiency. Therefore, it needs to be improved in the photo conversion efficiency and demonstrated the effective of KBNNOx-based to design up to the multi-functional devices in a certain property. The objective of the proposed research project is to design a new system of KBNNOx-based ceramic, which provides a good dielectric property and preserves a photovoltaic (PV) response. It has been known that the dielectric property relates to the oxygen vacancy, the $(1-x)[KNbO_3] - x[BaNi_{0.5}Nb_{0.5}O_{3-\delta}]$ with $x = 0.1$ is designed for reducing the effect of oxygen vacancy by setting $\delta = 0$, or called to KBNNO. The details of research are divided into subtopics: synthesis condition, atomic structure investigation, dielectric and PV properties, and effects of thickness and electrode on PV property. The importance of this research is that it will provide essential information to give better understanding of the details of advanced FE-PV materials, and will therefore inform, and provide guidelines for, the further development of high efficiency photo-converter devices, especially but not limited to a new type of PV cell.

Keywords: Solid-State Combustion Technique, X-ray Diffraction Spectroscopy, X-ray Absorption Spectroscopy, Raman Spectroscopy, Dielectric Property, Photovoltaic Property

Introduction

Smoking is the one of the main reasons of sickness and premature death global by using increasing educate publicly about the adverse effects of health and smoking in the workplace, However, the willpower, smokers using to try to quit have about 5 - 7% (Wongwiwatthananukit S., et al., 2009) for long term to success. Moreover, counseling behavior and medications using long term to quit rates in double or even trebling of the rates is successful approximately to quitting relative to placebo. Which is less than 30% generally (Wongwiwatthananukit S., et al., 2009), thus showing evidence that combination of counseling and medications resulted in a higher cessation rates than using either of them alone. Therefore, whenever feasible and appropriate, clinicians should encourage all patients attempting to quit to use effective medications for tobacco dependence treatment. The Guideline Panel identified seven first-line (FDA-approved) medications (bupropion SR, nicotine gum, nicotine inhaler, nicotine lozenge, nicotine nasal spray, nicotine patch, varenicline) and two second-line medications (clonidine and nortriptyline) as being effective for treating smokers. (Kitikannakorn N., et al., 2013.) Some medications are also associated with in unrequire side effects such as weight gain, nausea, dry mouth and sedation. (Wongwiwatthananukit S., et al., 2009) Moreover, there is clearly a need to search for alternative or new treatment for smoking cessation. Vernonia cinerea (VC), has been documented and widely used as a Thai traditional medicine and in other countries for relieving cigarette craving, asthma, cough, fever, malaria, arthritis and urinary calculi. (Wongwiwatthananukit S., et al., 2009) An infusion tea bag of a 4-gram which contained the whole dried crushed plant of VC 2.86 grams was prepared for relieving smoking cessation. (Wongwiwatthananukit S., et al., 2009)

Drug delivery systems for example mucoadhesion drug is conventional delivery methods to extend the drug at the section of application, permeability of the mucus membranes that allow rapid uptake of a drug into the systemic circulation. The ability of bioadhesions, determines to adhere to the mucus gel layer. (Lee, et al., 2000). They have been made earlier to formulate various forms, such as tablets (Boyapally, et al., 2010), films (Pongjaryakui and Suksri, 2009), patches (Morrow, et al., 2010), disks (Darwish, et al., 2008) and strips (Dixit and Puthil, 2009). Natural polysaccharides have been widely used as bioadhesive polymers because of their biocompatibility and biodegradability properties. CS (1-4, 2-amino-2-deoxi-beta-D-glucana) is a deacetylated derivative from the biopolysaccharide chitin. While chitin is considered an abundant and undesirable polysaccharide, the CS has shown an excellent biocompatibility, almost no toxicity to human beings, high bioactivity, biodegradability, reactivity of the deacetylated amino group, selective permeability, polyelectrolyte action, ability to form gel and film, chelation ability and absorptive capacity. (Kaur A., Kaur G., 2012) It was used as bioadhesive polymers to increase the residence time of the dosage form in buccal cavity. These polymers swell in aqueous media to form a gel through which the drug has to diffuse, so that they can also be used to control the rate of drug release. The release from CS by using herbal medicine, it has never done before. The aim of this study VC tea bag which hard to use and portable for smoker cessation combine with CS to be bioadhesive buccal patch. Thus the CS patch were designed by using appropriated thickness and formulations. In the present study, an attempt has been made to develop buccal patches of VC using CS

Material and Methods

Chitosan (CS) derived from crab shell, was purchased from Otta Chemika-Biochemika reagents, India. Reference standard beta- sitosterol was procured from Sigma Aldrich (Germany). Vernonia cinerea tea bag (VC) 2 and 4 mg per each bag and was gifts sample from Defence Pharmaceutical Factory. Lactic acid and all other reagents and chemicals were of analytical grade.

1. Formation of CS patches

From 10 formulations, the casting were chosen 4 formulations are the best, 2.2 grams was dissolved in 45 ml of distilled water and then added 1% (v/v) lactic acid, in some formulations the plasticizer (propylene glycol and glycerin) was added 10% (v/v) each formulation by magnetic stirring for 24 h, while stirring continuously, a solid sticky mass obtained for 24 h. The admixture was kept at 37 °C for 48 h. Each mixture was poured in the plates was dried in oven at 40°C for 48 h. The dried films were cut into square pieces of sides 2x2 cm containing 46-70 mg square pieces of sides per patch. The patches were packed in an aluminum foil and stored in an airtight glass container to maintain the integrity and elasticity of the patches. The patch was characterized using Fourier transform infrared spectroscopy (FTIR) studies.

2. Preparation of bioadhesive buccal patches

Buccoadhesive patches were prepared by solvent casting method. *Vernonia cinerea* tea bag (2 and 4 mg per each tea bag) were soaked 50 ml in boiling water 70 °C. Leaved tea bag at 37 °C. CS 2.2 grams was dissolved in VC solution and added lactic acid solution 2% (v/v) by magnetic stirring for 24 h, while stirring continuously, a solid sticky mass obtained for 24 h. The admixture was kept at 37 °C for 48 h. Each mixture was poured in the plates was dried in oven at 40°C. for 48 h. The dried films were cut into square pieces of sides 2x2 cm containing 50-80 mg square pieces of sides per patch. The patches were packed in an aluminum foil and stored in an airtight

glass container to maintain the integrity and elasticity of the patches. Table 1 shows the composition of formulated buccal patches. The patches were characterized using Fourier transform infrared spectroscopy (FTIR).

3. Mass uniformity and folding endurance test

Mass uniformity of the patches was tested in 10 different randomly selected patches from each batch and the patch thickness was measured at five different randomly selected spots using a Vernier caliper Folding endurance of the patch was determined by repeatedly folding one patch at the same place till it broke or folded up to 200 times without breaking (Khur, et al., 2000).

4. Surface pH determination

The surface pH of the patch was determined. The patches were allowed to swell by keeping them in contact with 1 ml of distilled water for 2 h at room temperature, and pH paper (litmus paper) contact with the surface of the patch,

5. VC analysis

Instrumentation and Chromatographic Conditions

The VC content was determined HPLC in condition, a waters symmetry shield C18 column (150 x 4.6, 5 μm) was used for the analysis. The mobile phase comprising of methanol: acetonitrile in the ratio (30:70) v/v was filtered through a 0.45 μm membrane filter (Millipore) and degassed by sonication. The VC extraction was carried out by injecting 10 μL of mixture of standard solution of assay concentration of beta-sitosterol. Throughout the run and flow rate of 1.0 ml min^{-} was maintained. The column effluent was monitored at 210 nm with L-2400 series multi-wavelength UV Detector. Typical HPLC chromatograms of Vernonia cinerea Linn. and determine beta-sitosterol as a marker and the retention time at 4.45 min.

6. Beta-sitosterol content uniformity

Beta-sitosterol content uniformity was determined by dissolving the patch by homogenization in 100 ml of an isotonic phosphate buffer (pH 6.6) for 2 h with occasional shaking. Aliquot (5 ml) was withdrawn and diluted with isotonic phosphate buffer pH 6.6 up to 20 ml, and the resulting solution was filtered through a 0.45 mm Whatman filter paper. The beta-sitosterol content was then determined by using beta-sitosterol standard conditions.

7. Measurement of bioadhesive strength

The bioadhesive strength of the bioadhesive patches was evaluated by using a universal testing machine (UTM) Cometech, CQ-506B, the model membrane for the measurement of bioadhesive strength. The thickness of patch 0.05 ± 0.01 mm approximately. The mucosal membrane was pulled by UTM, the film from the mucosal surface was determined and force of adhesion was taken as a measure of the film from the mucosal surface was determined and force of adhesion was taken as a measure of bioadhesive strength.

Results and discussion

Characterization of CS with plasticizer and CS with VC

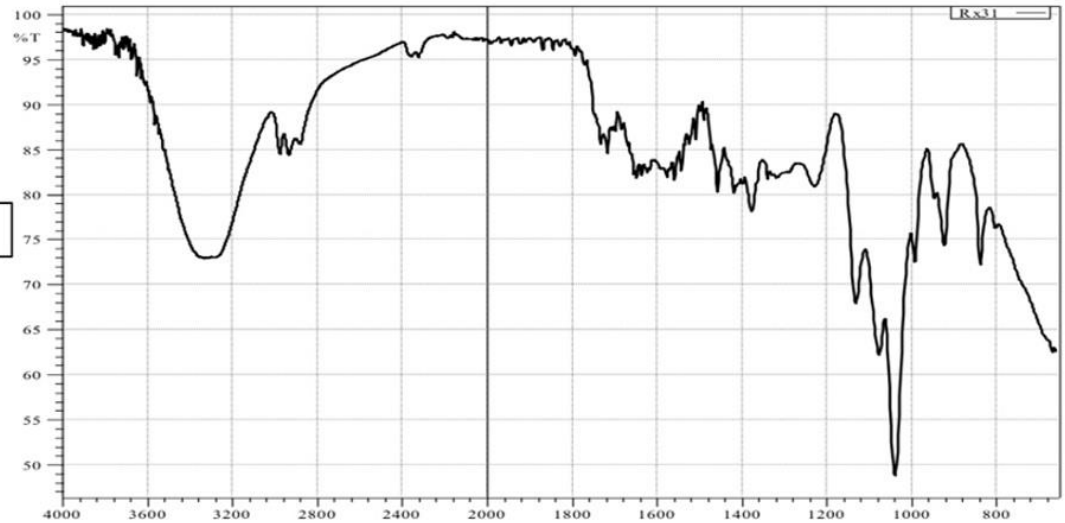
1. FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) analysis

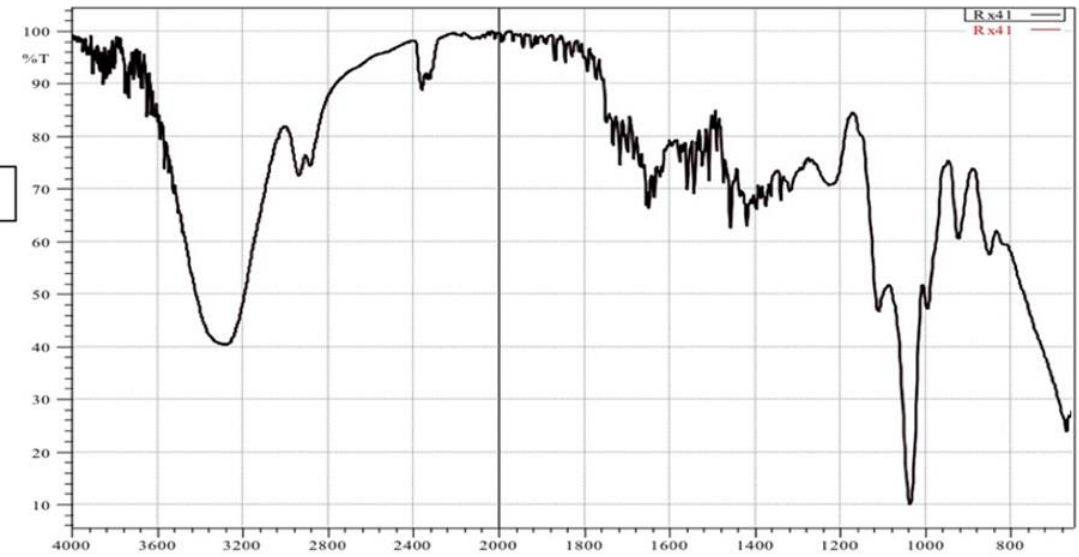
From composition of casting four formulations were showed in table 1. In wavelength Beta- sitosterol IR absorptions bands appeared at 3549.99 cm^{-1} (OH), 2935.73 cm^{-1} (CH2), 2867.38 cm^{-1} (CH), 1637.63 cm^{-1} (C=C), 1063.34 cm^{-1} (C-O) and other absorption peaks includes 1457.3 cm^{-1} (CH_2), 1381.6 cm^{-1}

(OH), 1038.7 cm^{-1} (cycloalkane) and 881.6 cm^{-1} (Figure1, Rx3, 4). The effect of the glycerin (glycerol,G) addition was also studied by FTIR analysis. The films infrared spectra are shown in Figure 1 (Rx1). In the mentioned wave number range, it was possible to observe two shifts from the G addition. The first one was the shift of the peak located at 936 cm^{-1} to 925 cm^{-1} after the G addition related to the symmetric stretching vibrations of the alcoxyl group. The second one was the shift of the peak corresponding to the symmetric stretching vibrations of the alcoxyl group (C-O-C) at 999 cm^{-1} to 993cm $^{-1}$ using the highest G concentration. For the significantly higher G content used in this work, in contrast, we found a shift to higher wave numbers: the peak was located at 2876.44 cm^{-1} and for the highest concentration at 2887.86 cm $^{-1}$. This could be related with the formation of glycerol clusters surrounding the chitosan molecules. As a consequence, the resulting area of the band grew with the G content indicating a higher intensity of hydrogen bonds which could be related with more OH groups provided by the G molecules (Figure 1, Rx1). Rx2 In order to confirm the CS-PG interactions were analyzed by FTIR spectroscopy showed in Figure1 (Rx2). FTIR spectra of CS-PG Showed peaks around 946 cm $^{-1}$ and 1068 cm^{-1} of assigned saccharine structure. Moreover the spectra of Rx2 are closed to Rx1, but some different peak from Rx1 (Kaur A, Kaur G., 2012).

a)Rx1



b)Rx2



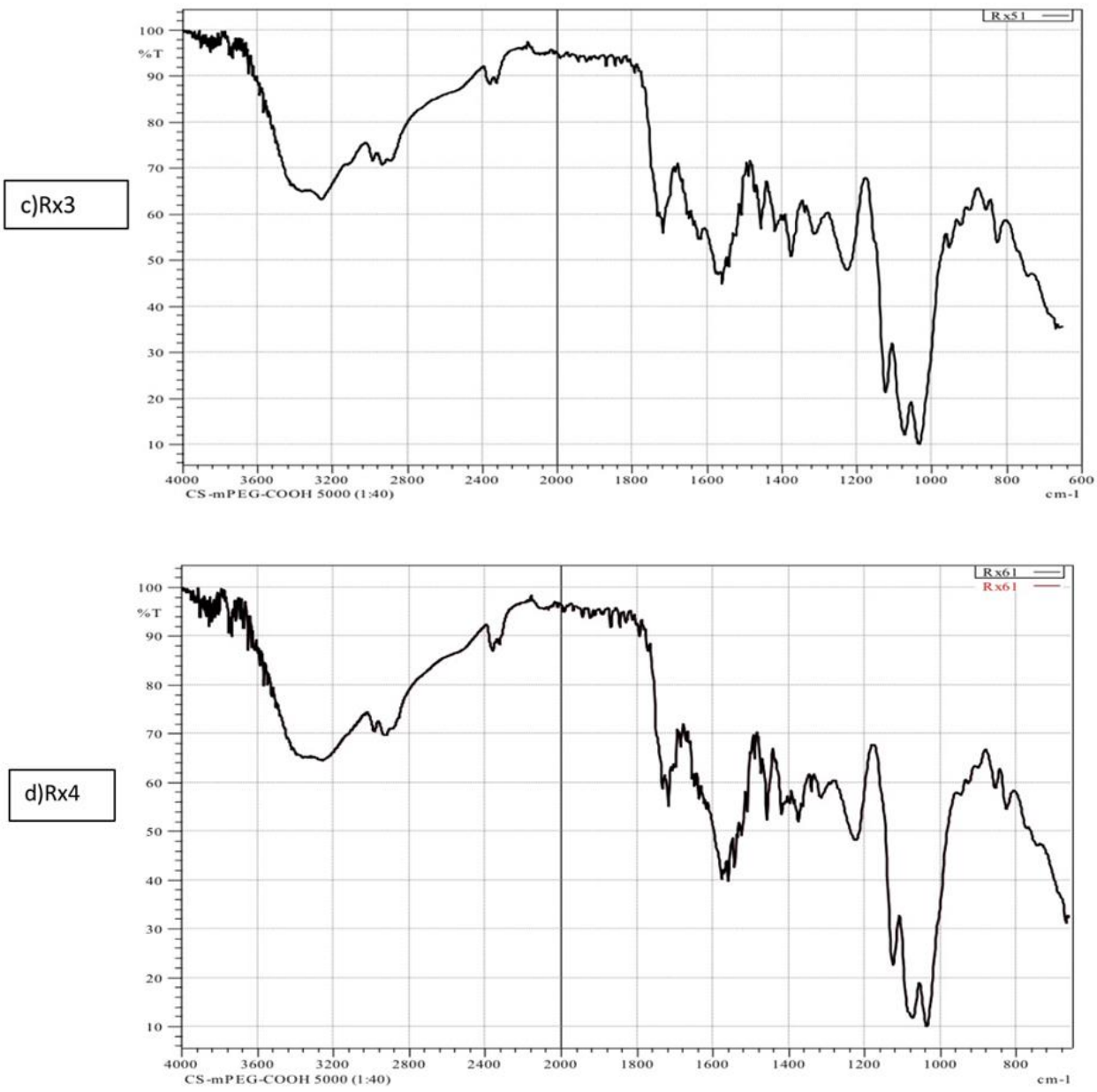


Figure 1 IR Spectrum a)Rx1 b)Rx2 c)Rx3 d)Rx4

Table 1 Composition of casting four formulations

No. of formulation	Chitosan (%w/v)	Lactic (%v/v)	Glycerin (%v/v)	Propylene glycol (%v/v)	CS:VC
Rx1	2	2	10	-	100:0
Rx2	2	2	-	10	100:0
Rx3	2	2	-	-	96:4
Rx4	2	2	-	-	92:8

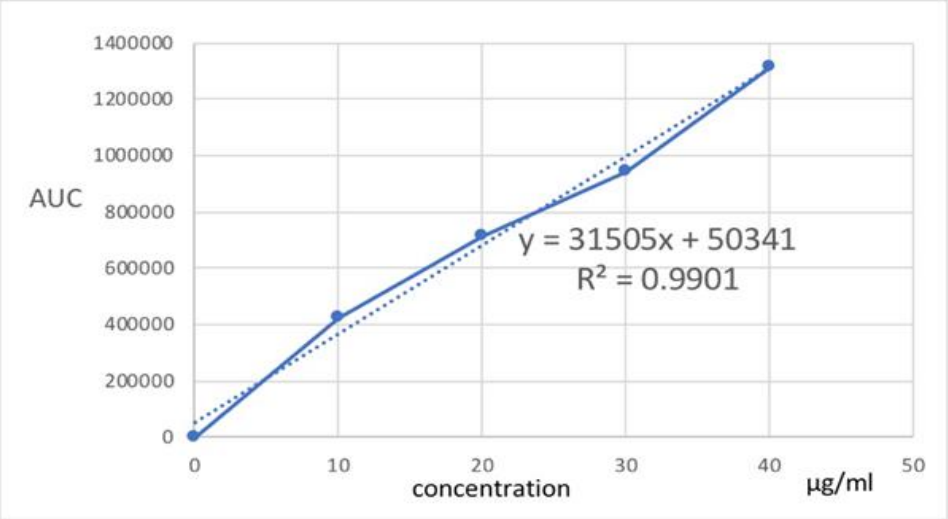
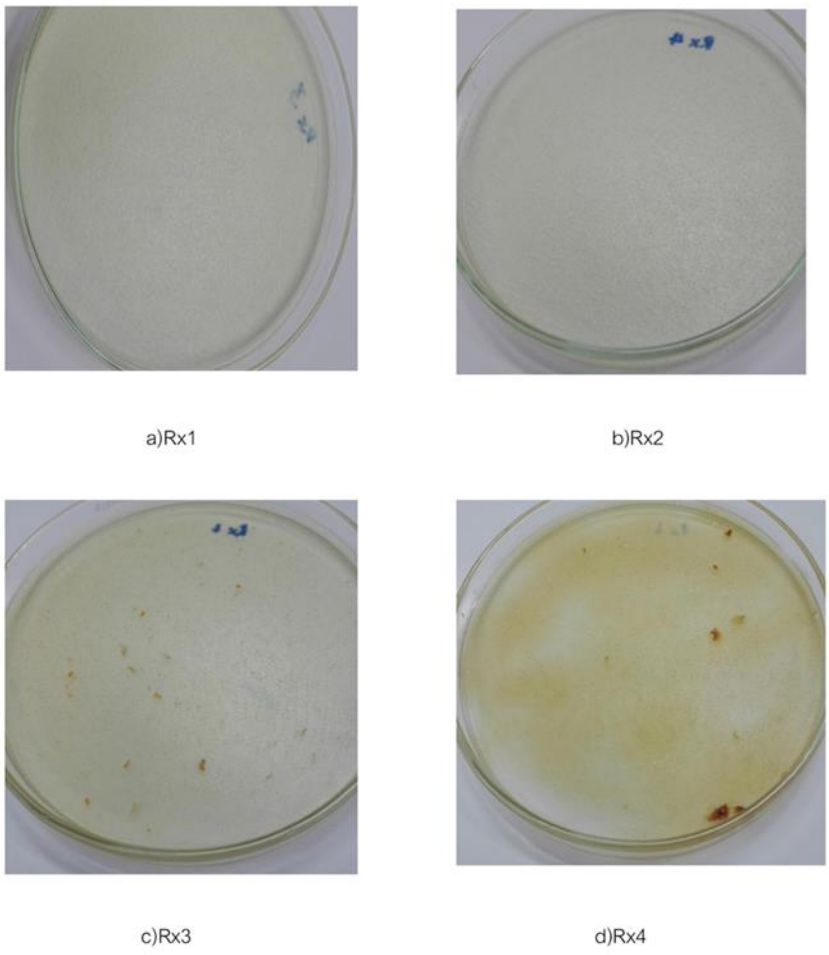


Figure 2 Standard curve of beta-sitosterol



a)Rx1 b)Rx2

c)Rx3 d)Rx4

Figure 3 Final formulations after oven at 40°C for 48 h

2. Physical characteristics of bioadhesive patches

The regression equations and correlation coefficient for the reference were y = 31505x + 50341, R^2 = 0.9901 for beta-sitosterol (figure 2). In table 2, the prepared patches were smooth in appearance (figure 3) compare to six formulations (figure4, table 4). Figure 3 showed uniform in thickness, mass and drug content and showed no visible cracks. Figure 4 was exhibited non uniformity of thickness and rough appearance. In table2, the patches were exhibited good folding endurance. The thickness of the patch ranged from 0.10±0.45 to 0.23±0.40 mm and mass exhibited in range from 59 ± 2 to 65±1mg The bioadhesive patches had a surface pH of 3.3 ± 0.10 to 3.5 ± 0.2 and the VC contents (Rx3, Rx4) in buccal patches were found to range of Beta-sitosterol from 1.48± 2% to 1.52 ± 3%

Table 2 Physical characteristics of bioadhesive Vernonia cinerea patch.

Patch code	Mass (mg)	Thickness (mm)	beta-sitosterol content (%) (g/100g)	F = Folding endurance (F = log10 *d*, *d* =double fold)	Surface pH
Rx1	59±2	0.10±0.45	-	74±12	3.3±0.1
Rx2	58±3	0.11±0.54	-	68±10	3.3±0.2
Rx3	64±1	0.20±0.34	1.48±2	153±14	3.4±0.1
Rx4	65±1	0.23±0.40	1.52 ±3	155±17	3.5±0.2

All the experiments were carried out in triplicate.

3. Bioadhesive strength studies

The patches formulated using CS and plasticizer glycerin and PG (Rx1 and Rx2) were showed no stress in table 3 bioadhesive strength. Glycerin and PG being hydrophilic in nature forms a gel like structure resulting no stress. However, an increase in the bioadhesive strength was observed in Rx3 and Rx4. This observation can be explained by the presence of CS in the cationic (protonated) form with VC which showed stress in MPa digit. This led to electrostatic interactions between CS and negatively charged mucus (Kaur, 2012) thus resulting in increased bioadhesive strength as compared to CS alone (Rx1, Rx2).

Table 3 Measurement of bioadhesive stress

	Sample	Sample	Maximum	Max Load	Max Load	Max Load	Max Load	Secant	Break	Break
	Thickness	Width	Load	Displace	Stress	Strain	Elong	Modulus	Stress	Elong
Unit/ No.	mm	mm	N	mm	MPa	strain		N/mm	MPa	MPa
Rx1	0.10	10	0.67	5.50	NA	NA	NA	NA	NA	NA
Rx2	0.11	10	0.75	6.00	NA	NA	NA	NA	NA	NA
Rx3	0.20	10	2.21	6.03	1.1032	0.2	20.11	0.4054	0.3334	21.82
Rx4	0.24	10	1.03	5.47	0.6865	0.18	18.23	0.1994	0.2092	21.78
Mean (Rx3-Rx4)	0.175	10	1.62	5.752	0.8948	0.19	19.17	0.3024	0.2713	21.8
Std (Rx3-Rx4)	0.035	0	0.83	0.399	0.2947	0.01	1.33	0.1457	0.0878	0.03

NA Cannot detect

All the experiments were carried out in triplicate.

Table 4 Composition of CS-VC formulations oven at various temperature

Formulation	CS(g)	VC(g)	Glycerin(ml)	Temperature(˚C)
Rx5	1.10	3	-	75
Rx6	2.20	-	-	40
Rx7	1.10	-	5	40
Rx8	1.10	3	-	40
Rx9	1.10	-	-	40
Rx10	1.10	3	-	60

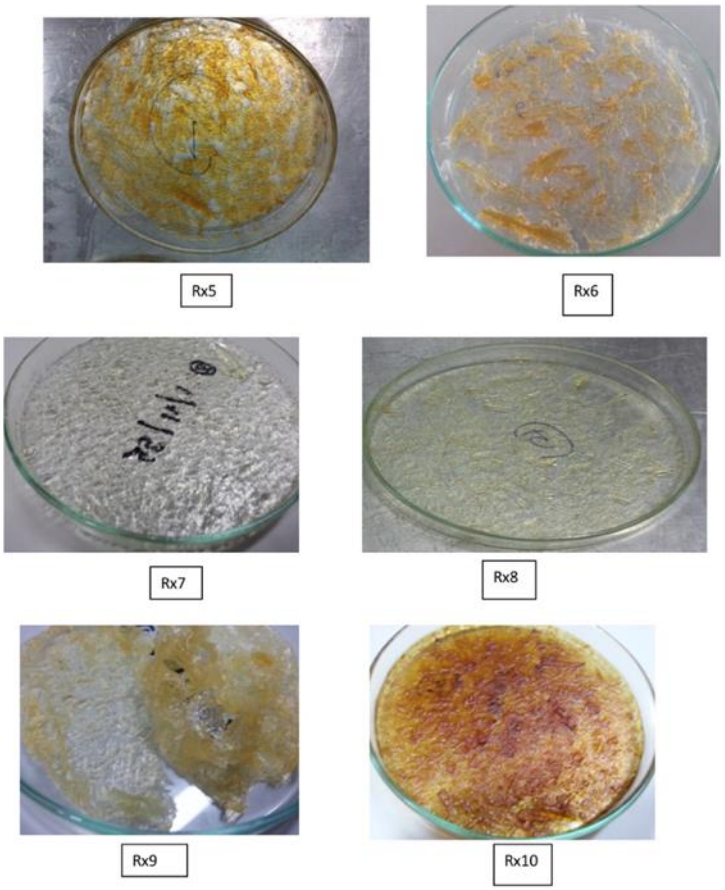


Figure 4 Six formulations after various compositions and various oven

Conclusion

In the present investigation Rx1-Rx4 were prepared and the physicochemical interaction between CS and VC was investigated by FTIR. The patches were evaluated for their physical characteristics like mass variation, content uniformity, folding endurance and bioadhesive stress. The surface pH of all the formulated bioadhesive patches was found between 3.3 to 3.5. On the basis of the above results it can be concluded that CS-VC from 5 g of tea bag (Rx4) which the proper formulate bioadhesive buccal patches of VC and it should be observed the release for further study.

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