

# Effect of Biofield Treatment on Physical, Thermal, and Spectral Properties of SFRE 199-1 Mammalian Cell Culture Medium

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## To cite this article:

Mahendra Kumar Trivedi, Alice Branton, Dahryn Trivedi, Gopal Nayak, Khemraj Bairwa, Snehasis Jana. Effect of Biofield Treatment on Physical, Thermal, and Spectral Properties of SFRE 199-1 Mammalian Cell Culture Medium. *Advances in Biochemistry*.

Vol. 3, No. 6, 2015, pp. 77-85. doi: 10.11648/j.ab.20150306.13

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**Abstract:** SFRE 199-1 medium (SFRE-M) is important mammalian cell culture medium, used for the culture of primary cells of mammals such as baboon kidney cells. The present study was attempted to evaluate the impact of biofield energy treatment on the physical, thermal and spectral properties of SFRE-M. The study was accomplished in two groups; one was set as control while another was subjected to Mr. Trivedi's biofield energy treatment and coded as treated group. Subsequently, the control and treated samples were analyzed using various analytical techniques. The CHNO analysis showed about 2.16, 4.87, and 5.89% decrease in percent contents of carbon, hydrogen, and oxygen, respectively; while 9.49% increase in nitrogen contents of treated sample as compared to the control. X-ray diffraction (XRD) analysis showed 7.23% decrease in crystallite size of treated sample as compared to the control. The thermogravimetric analysis (TGA) analysis showed the increase in onset temperature of thermal degradation by 19.61% in treated sample with respect to the control. The control sample showed the 48.63% weight loss during the thermal degradation temperature ( $T_{max}$ ) while the treated sample showed only 13.62% weight loss during the  $T_{max}$ . The differential scanning calorimetry (DSC) analysis showed the 62.58% increase in the latent heat of fusion of treated sample with respect to the control sample. The Fourier transform infrared spectroscopy (FT-IR) spectrum of treated SFRE-M showed the alteration in the wavenumber of C-O, C-N and C-H vibrations in the treated sample as compared to the control. Altogether, the XRD, TGA-DTG, DSC, and FT-IR analysis suggest that Mr. Trivedi's biofield energy treatment has the impact on physical, thermal and spectral properties of SFRE-M. The treated SFRE-M was more thermal stable than the control SFRE-M and can be used as the better culture media for mammalian cell culture.

**Keywords:** Biofield Energy Treatment, SFRE-Medium, Elemental Analysis, X-ray Diffraction, Fourier Transform Infrared Spectroscopy

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## 1. Introduction

Medium M-199 is a well-defined nutritional source for cell culture media, developed in 1950 by Morgan *et al.* [1, 2]. It is the combinations of amino acids, vitamins and other factors, which exhibited the best growth of explanted tissue in the *in vitro* studies [3]. It was initially designed for nutritional studies to promote the growth of primary chick embryo heart and fibroblast cells in the absence of serum supplement [3, 4]. Although, the M-199 medium is also useful with serum for growth of a wide range of cell types such as non-

transformed chicken, monkey, and human cells. The SFRE 199-1 medium (SFRE-M) is the modified form of medium 199, and developed for the growth and maintenance of mammalian cell culture (such as primary baboon kidney cells) [5]. It is obtained by supplementing the medium M-199 with sodium pyruvate, zinc sulfate, and increasing arginine-HCl, cystine, cysteine, L-glutamic acid, L-glutamine, glycine, tyrosine, histidine, and glucose to maximally active nontoxic concentrations [6].

Sterilization process plays a significant role on the quality of culture media. The autoclaving (heat treatment) is the principle method of culture media sterilization [7]. This heat

treatment of complex culture media may result to nutrient destruction via the direct thermal degradation or by the chemical reactions between the components [8]. Therefore, an alternate method is required, which can enhance the overall stability of the culture media such as SFRE-M [9].

Recently, the energy healing therapies have been reported for several beneficial effects throughout the world. Biofield energy treatment is one of the energy therapies that has been reported to alter the various physicochemical properties of organic compounds [10] and organic products [11].

The energy medicines have been categorized by National Center for Complementary and Alternative Medicine (NCCAM) under the CAM therapies [12]. There are several proposed mechanism and explanations are offered to support the biofield energy therapies. Consciousness is one of the possible mechanisms, which includes healer's intent to heal, may interact with the physical realm [13]. Likewise, physical resonance is another theory that includes subtle energies. According to this theory, the energy might be exchange between the energy fields of healer and patient [14]. The healer or practitioner of energy medicine harness the energy from universe and transmit it to the object (living or non-living), which is called biofield energy treatment. Mr. Trivedi is a renowned practitioner of energy medicine and his unique biofield energy treatment is known as The Trivedi Effect<sup>®</sup> that has been studied in the field of agricultural science research [15], biotechnology research [16], and microbiology research [17], etc.

After conceiving the significant impact of biofield energy treatment in different field of science, the present study was attempted to evaluate its impact on the culture media such as SFRE-M culture media. The biofield energy treated SFRE-M was analyzed along with the control sample using several analytical techniques like elemental (CHNO) analysis, X-ray diffractometry (XRD), thermogravimetric analysis-derivative thermogravimetry (TGA-DTG), DSC, and Fourier transform infrared (FT-IR) spectroscopy. The data of treated sample was compared with that of control as well as reported literature data.

## 2. Materials and Methods

### 2.1. Study Design

The SFRE 199-1 media (SFRE-M) was procured from HiMedia Laboratories, India. It consists with several inorganic salts, vitamins, amino acids, sugars, etc. (Table 1). The SFRE-M was divided into two groups; one was kept as control (without treated) while another was handed over to Mr. Trivedi to render the biofield energy treatment under laboratory conditions. Mr. Trivedi provided the biofield energy treatment to the treated group *via* his unique energy harnessing process through the thought transmission (without touching the sample). Then, both the control and treated samples were explored with respect to physicochemical and spectroscopic properties using various techniques like elemental (CHNO) analysis, XRD, TGA-DTG, FT-IR and

UV-vis spectroscopy.

**Table 1.** Chemical composition of SFRE 199-1 medium.

Ingredients	mg/L	Ingredients	mg/L
<b>INORGANIC SALTS</b>		<b>VITAMINS</b>	
Sodium chloride	8000.000	Choline chloride	0.500
Potassium chloride	400.000	Retinol Acetate	0.140
Calcium chloride dihydrate	185.000	Calciferol	0.100
Magnesium sulphate anhydrous	97.720	L-Ascorbic acid	0.050
Potassium phosphate monobasic	60.000	i-Inositol	0.050
Sodium acetate	50.000	p-Amino benzoic acid (PABA)	0.050
Sodium phosphate dibasic anhydrous	47.860	Niacin + Niacinamide (1:1)	0.050
Ferric nitrate nonahydrate	0.720	Pyridoxal hydrochloride	0.025
Zinc sulphate heptahydrate	0.100	Pyridoxine hydrochloride	0.025
<b>AMINO ACIDS</b>		Menadione sodium bisulphite	0.016
L-Glutamine	300.000	Folic acid + Riboflavin (1:1)	0.020
L-Arginine hydrochloride	150.000	D-Ca-Pantothenate	0.010
L-Tyrosine disodium salt	116.000	D-Biotin	0.010
Glycine	100.000	DL-Tocopherol phosphate disodium salt	0.010
L-Glutamic acid	75.000	Thiamine hydrochloride	0.010
L-Lysine hydrochloride	70.000	OTHERS	
L-Leucine	60.000	D-Glucose	2000.000
L-Cystine dihydrochloride	43.800	D-(+)-Galactose anhydrous	1000.000
L-Proline	40.000	Sodium pyruvate	150.000
L-Histidine hydrochloride monohydrate	40.000	Adenine sulphate	10.000
L-Aspartic acid	30.000	Phenol red	10.000
L-Threonine	30.000	Polysorbate 80	4.900
L-Alanine	25.000	Adenosine triphosphate disodium	1.000
L-Phenylalanine	25.000	Deoxyribose + Ribose (1:1)	1.000
L-Serine	25.000	Hypoxanthine	0.354
L-Valine	25.000	Xanthine	0.344
L-Isoleucine	20.000	Guanine hydrochloride	0.300
L-Methionine	15.000	Thymine +Uracil (1:1)	0.600
Hydroxy-L-Proline 1	10.000	Adenosine monophosphate	0.200
L-Tryptophan	10.000	Cholesterol	0.200
L-Cysteine (free base)	4.000	Glutathione reduced	0.050

### 2.2. Elemental (CHNO) Analysis

The control and treated samples of SFRE-M were analyzed for their elemental composition, carbon (C), hydrogen (H), nitrogen (N), oxygen (O), and sulfur (S). The analysis was done using Model Flash EA 1112 Series, Thermo Finnigan Italy. This analyzer combusts and then

converts the C, H, N and O sample elements to simple gases, *i.e.* CO<sub>2</sub>, H<sub>2</sub>O, N<sub>2</sub>, and O<sub>2</sub>, which were determined quantitatively to deduce the net contents of respective element in the sample. The percentage change in element of the treated sample with respect to the control was determined using the following equation.

$$\% \text{ Change in element (C, H, N, or O)} = [(E_T - E_C) / E_C] \times 100$$

Here, E<sub>T</sub> and E<sub>C</sub> are the element in control and treated samples, respectively.

### 2.3. XRD Study

The XRD analysis of control and treated samples of SFRE-M was done on Phillips (Holland PW 1710) X-ray diffractometer with copper anode and nickel filter. The wavelength of XRD instrument was set to 1.54056Å. The

percent change in crystallite size (G) was calculated using following equation:

$$G = [(G_T - G_C) / G_C] \times 100$$

Here, G<sub>T</sub> and G<sub>C</sub> are average crystallite size of treated and control samples, respectively.

### 2.4. TGA-DTG Analysis

The TGA-DTG analysis of control and treated sample was carried out on Mettler Toledo simultaneous TGA-DTG analyzer. The analytes were heated up to 400°C from room temperature at the heating rate of 5°C/min under air atmosphere. The onset temperature of thermal degradation and temperature at which maximum weight loss occur (T<sub>max</sub>) in samples were obtained from TGA-DTG thermogram.

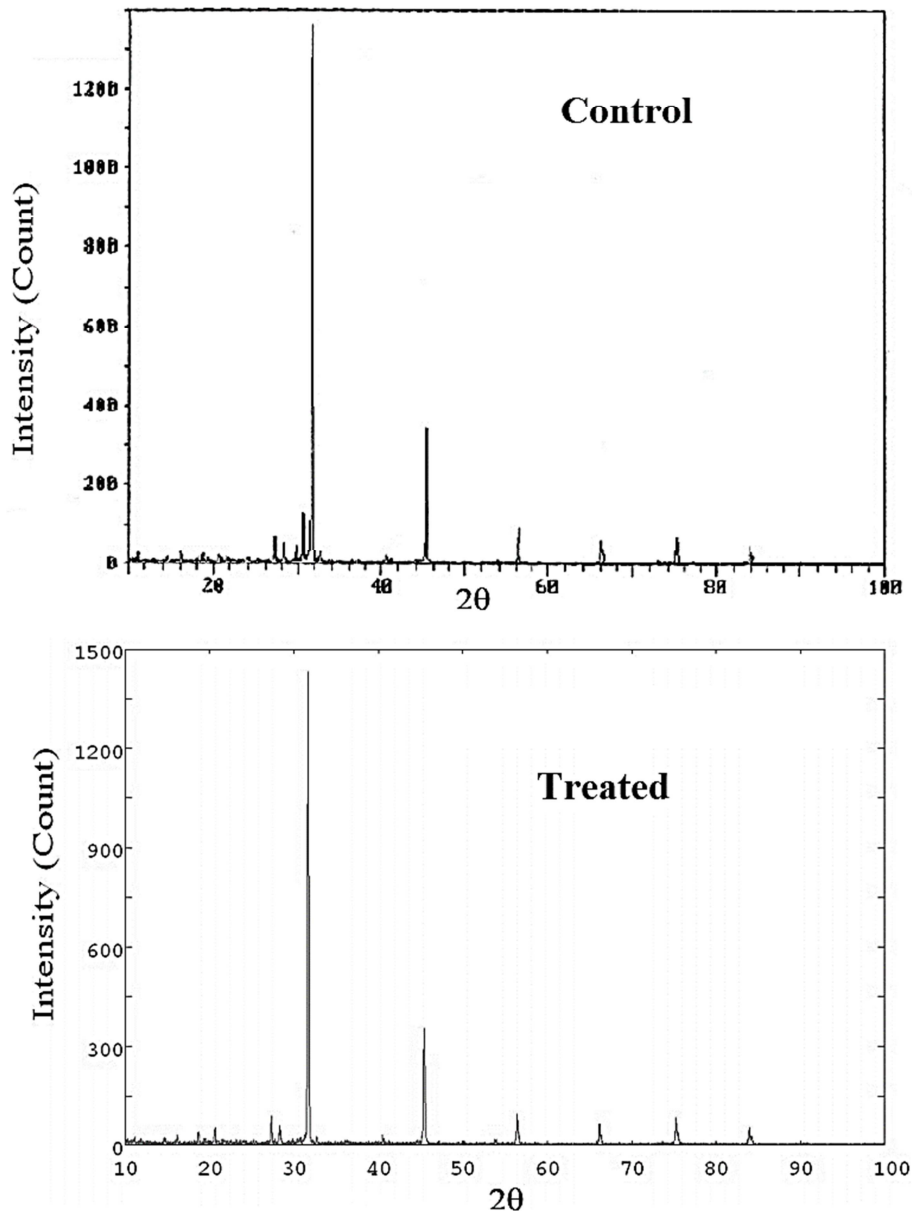


Fig. 1. XRD diffractograms of control and treated SFRE 199-1 medium.

### 2.5. DSC Study

The melting temperature and latent heat of fusion of control and treated SFRE-M were determined using the Pyris-6 Perkin Elmer differential scanning calorimeter. The samples were heated upto 300°C at the heating rate of 10°C/min under air atmosphere with air flow rate of 5 mL/min.

### 2.6. FT-IR Spectroscopic Characterization

The FT-IR spectroscopy was carried out to evaluate the effect of biofield energy treatment on force constant and bond strength in chemical structure [18]. The samples for FT-IR analysis were prepared with spectroscopic grade KBr into pellets. The spectra were recorded on Shimadzu's Fourier transform infrared spectrometer (Japan) with the frequency array of 500-4000 cm<sup>-1</sup>.

## 3. Results and Discussion

### 3.1. Elemental (CHNO) Analysis

The elemental analysis is used to quantify the percent content of elements present in the organic compounds or products. The impact of biofield energy treatment on C, H, N, and O is reported in Table 2. The result showed that content of carbon, hydrogen and oxygen were decreased with 2.16, 4.87, and 5.89%, respectively in the biofield treated SFRE-M as compared to the control. On the other hand, the content of nitrogen element was increased significantly by 9.49% in treated sample with respect to the control SFRE-M.

**Table 2.** CHNO analysis data of control and treated SFRE 199-1 medium.

Element	Control	Treated	% Change
Carbon	13.173	12.889	-2.16
Hydrogen	2.135	2.031	-4.87
Nitrogen	1.054	1.154	9.49
Oxygen	12.40	11.67	-5.89

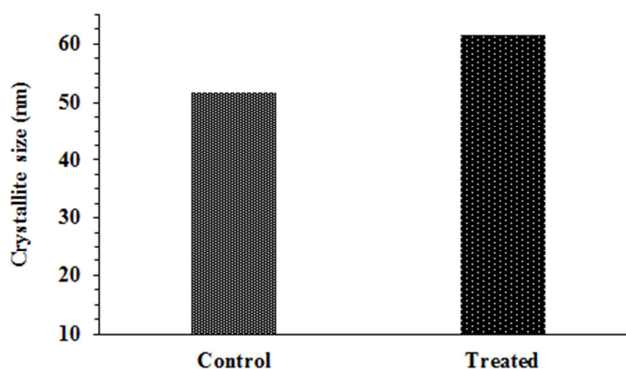
Although, the mammalian cell culture require numerous essential components, but the nitrogen content is the principal requirement for the proper growth and development of primary mammalian cells [19, 20]. There are several nitrate salts and amino acids used as the source of nitrogen in the culture medium [21]. In the present study, the nitrogen content was increased significantly in the treated sample as compared to the control. This might be useful for the better growth of mammalian cells as compared to the control SFRE-M.

### 3.2. XRD Analysis

The XRD diffractograms of SFRE-M (control and treated) samples are shown in Fig. 1. The XRD diffractograms of both the samples (*i.e.* control and treated SFRE-M) showed the sharp and intense peaks, which suggest the crystalline nature of both samples. The XRD diffractogram of control

SFRE-M showed the peaks at Bragg's angle ( $2\theta$ ) equal to 27.24°, 28.24°, 29.63°, 30.55°, 31.52°, and 45.26°. Similarly, the XRD diffractogram of treated SFRE-M exhibited the XRD peaks at  $2\theta$  equal to 27.33°, 28.34°, 31.67°, 32.66°, 40.48°, and 45.41°.

The XRD analysis showed the crystallite size of the control and treated samples as 51.72 and 61.45 nm, respectively. The result showed 18.82% increase in the crystallite size of treated sample as compared to the control (Fig. 2).



**Fig. 2.** Crystallite size of control and treated SFRE 199-1 medium.

It is assumed that biofield energy might induce some internal strain in the treated molecules and resulted in the re-orientation of neighboring planes, which is also called as atomic displacement [22, 23]. This might lead to increase the crystallite size in the treated sample.

### 3.3. TGA-DTG Analysis

The TGA-DTG thermogram of SFRE-M samples (control and treated) are shown in Fig. 3 and data are presented in Table 3.

The TGA thermogram of control sample exhibited the initiation (onset) of thermal degradation at about 102°C, which was ended (endset) at about 221°C. Likewise, the TGA thermogram of the treated SFRE-M exhibited the initial (onset) thermal degradation temperature at about 122°C that was terminated (endset) at about 200°C.

**Table 3.** Thermal analysis of control and treated samples of SFRE 199-1 medium.

Parameter	Control	Treated
Onset temperature (°C)	102.00	122.00
Endset temperature (°C)	221.00	200.00
T <sub>max</sub> (°C)	162.00	156.00
Latent heat of fusion (J/g)	44.79	72.82
Melting point (°C)	145.76	149.83

T<sub>max</sub>: temperature at maximum weight loss occurs

The result indicated about 19.61% increase in the onset temperature of thermal degradation in biofield energy treated sample with respect to the control. Moreover, the maximum thermal degradation temperature (T<sub>max</sub>) of control sample

was observed at about 162°C with about 48.63% weight loss; while the treated sample showed the  $T_{max}$  at 156°C with only 13.62% weight loss. This indicated that the percentage of weight loss during the thermal degradation was less in the treated sample with respect to the control. It suggests the possible increase in the thermal stability of treated sample as

compared to the control [24,25]. Further, the result showed about 3.70% decrease in  $T_{max}$  of treated SFRE-M as compared to the control. This might be occurred due to the alteration in internal energy *via* biofield energy treatment, which may lead to early phase of evaporation in treated sample as compared to the control [26].

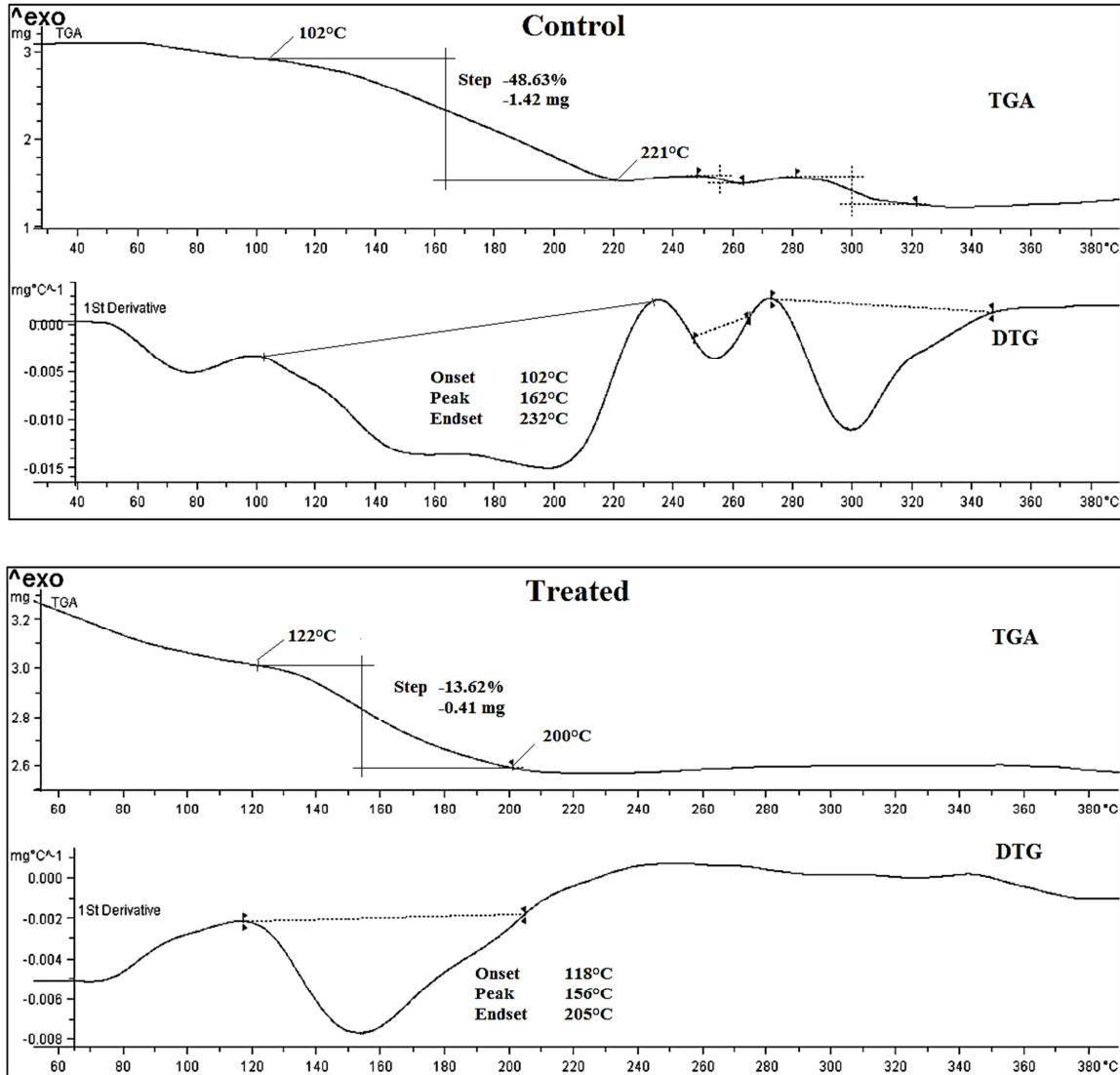


Fig. 3. TGA-DTG thermograms of control and treated SFRE 199-1 medium.

### 3.4. DSC Analysis

DSC analysis was performed to determine the melting temperature and latent heat of fusion ( $\Delta H$ ) of control and treated TPP samples. A substantial amount of interaction force is present in the atomic bonds of any substance that hold the atoms at their positions. The energy required to overcome the interaction force of phase change *i.e.* solid into liquid, is called as the  $\Delta H$ . DSC thermogram (Fig. 4) of SFRE-M showed the melting temperature at 145°C in control and 149.83°C in treated sample (Table 2).

The result showed about 2.79% increase in the melting temperature of the treated sample of SFRE-M as compared to

the control. Moreover, the DSC thermogram exhibited the latent heat of fusion *i.e.* 44.79 J/g in control and 72.82 J/g in the treated sample of SFRE-M. The result showed about 62.58% increase in the latent heat of fusion of treated sample with respect to the control sample. This might be due to increase in intermolecular force in the treated SFRE-M with respect to the control. As a result, the treated SFRE-M sample probably required more energy ( $\Delta H$ ) to change the phase from solid to liquid as compared to the control. Formerly, our group has reported that biofield energy treatment caused the changes in  $\Delta H$  in lead and tin powders [27]. Therefore, it is supposed that biofield treatment might alter the intermolecular interaction forces of treated SFRE-M molecules, which may lead to alteration in latent heat of fusion.

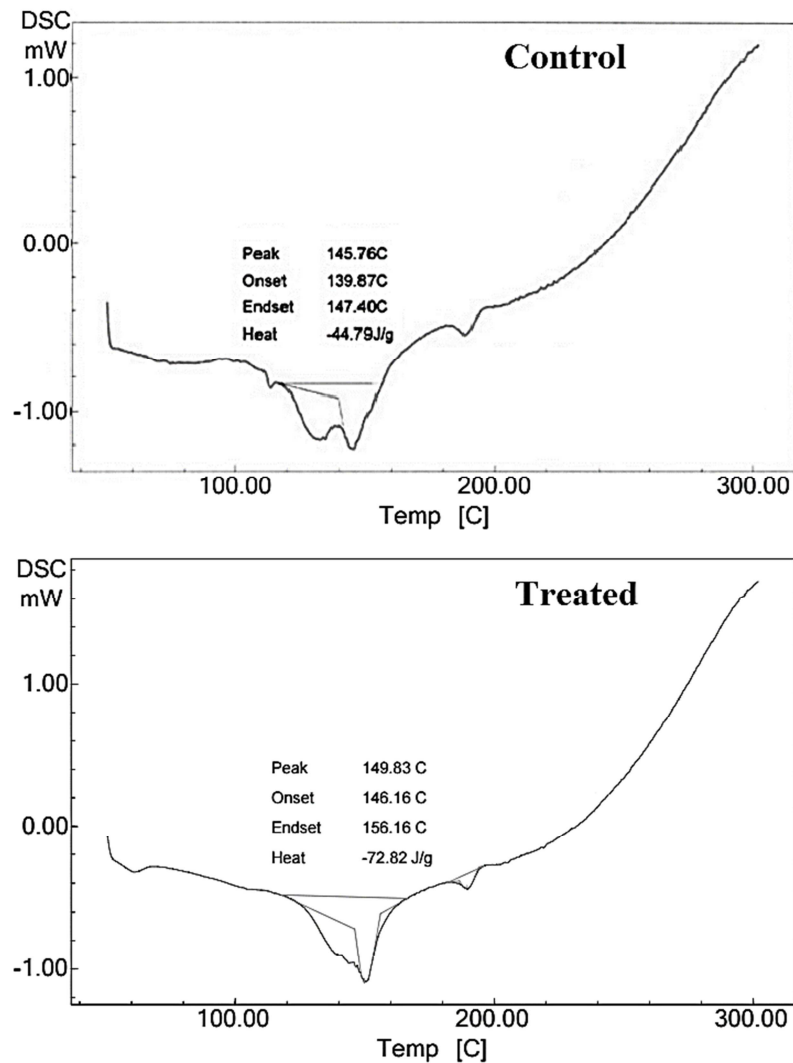


Fig. 4. DSC thermograms of control and treated SFRE 199-1 medium.

### 3.5. FT-IR Spectroscopic Analysis

FT-IR spectra of the control and treated SFRE 199-1 M are shown in Fig. 5. The SFRE-M molecule contains O-H, N-H, C-H, C=C, C=O, C-C, C-Cl groups of vibrations. A broad peak in the frequency array of 2900 to 3600  $\text{cm}^{-1}$  in both the control and treated samples may be due to O-H or N-H stretching. The vibrational peak appeared in the region of 2817-2893  $\text{cm}^{-1}$  in the control sample that might be due to the C-H stretching. This frequency region was shifted in the treated sample and observed at 2833-2902  $\text{cm}^{-1}$ , which indicated a possible increase in the bond strength of C-H group in the treated sample with respect to the control. The IR peaks observed at 1629  $\text{cm}^{-1}$  (control) and 1627  $\text{cm}^{-1}$  (treated) were might be attributed to the amide or acidic C=O group of amino acids present in the SFRE-M. Vibrational peak at 1411  $\text{cm}^{-1}$  in control and treated sample might assign to C-H bending.

The peak observed at 1355  $\text{cm}^{-1}$  in control sample and 1359  $\text{cm}^{-1}$  in treated sample were possibly due to the N=O symmetric stretching in amino acid. Likewise, the IR peak at

1326  $\text{cm}^{-1}$  in control and 1328  $\text{cm}^{-1}$  in the treated sample was possibly due to the S=O stretching of sulfate salts, present in the SFRE-M. Moreover, the peaks at 1247  $\text{cm}^{-1}$  (control) and 1244  $\text{cm}^{-1}$  (treated) were might be due to C-C stretching. The vibrational peak appeared at 1024 and 1149  $\text{cm}^{-1}$  in control sample were might be due to C-O stretching. This was appeared at slightly downstream region *i.e.* at 1022 and 1141  $\text{cm}^{-1}$  in the treated sample, which indicated a possible decrease in the bond strength of C-O bond in the treated sample with respect to the control. The vibrational peak at 1047  $\text{cm}^{-1}$  in control sample, which might be due to C-N stretching that was shifted to upstream region of IR frequency *i.e.* at 1060  $\text{cm}^{-1}$  after the biofield treatment. It suggested that the force constant of C-N bond in the treated sample was possibly increased as compared to the control. Likewise, the peaks at 848  $\text{cm}^{-1}$ , 769  $\text{cm}^{-1}$ , and 623  $\text{cm}^{-1}$  in control sample were might be attributed to aromatic breathing, S-N stretching and metal-halide stretching, respectively. These peaks were correspondingly appeared at 846  $\text{cm}^{-1}$ , 767  $\text{cm}^{-1}$ , and 617  $\text{cm}^{-1}$  in the treated sample.

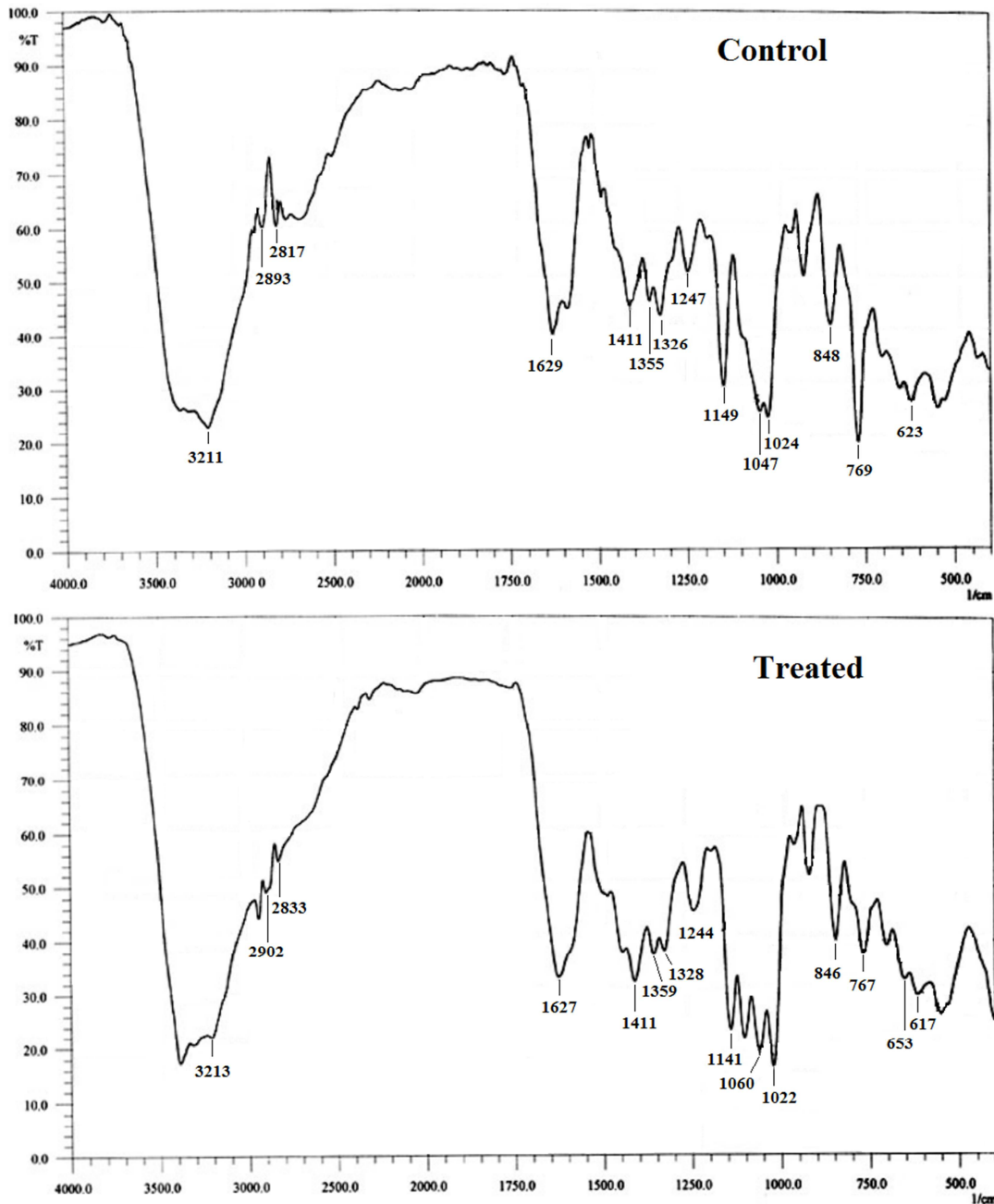


Fig. 5. FT-IR spectra of control and treated (T1 and T2) SFRE 199-1 medium.

Frequency ( $\nu$ ) of stretching vibrational peak is mainly depends on two factors *i.e.* force constant ( $k$ ) and reduced mass ( $\mu$ ), which can be explained by following equation [18, 28]  $\nu = 1/2\pi c \sqrt{k/\mu}$ ; here,  $c$  is the speed of light.

At the constant  $\mu$ , the frequency is directly proportional the force constant [29]. Based on this, it is presumed that force constant of some bond such as C-H and C-N might increase in the treated sample, which might lead to increase in the stability of treated molecules with respect to the control. The thermal stability data was also support the increased stability of treated SFRE-M with respect to the control.

## 4. Conclusion

In conclusion, the present study showed the substantial alteration in the percent of C, H, N, and O element of treated sample by 2.16, 4.87, 9.49, and 5.89%, respectively as compared to the control sample. The XRD study suggested the crystalline nature of both the control and treated samples. Moreover, the crystallite size of treated sample was decreased by 18.82% with respect to the control sample. The TGA-DTG study showed about 19.61% increase in the initiation temperature of thermal degradation; while the

percent weight loss during the maximum thermal decomposition temperature was also reduced significantly in the treated sample with respect to the control. This showed the increase in the thermal stability of treated SFRE-M as compared to the control. The DSC analysis showed the increased in the melting temperature (2.79%) and latent heat of fusion (62.58%) of treated sample with respect to the control. The FT-IR data showed the alteration in the vibrational frequency of some groups like C-H, C-O, and C-N in treated sample with respect to the control. This might be due to the increase in force constant and bond strength of respective groups in treated SFRE-M molecule as compared to the control.

Overall, the data suggest that Mr. Trivedi's biofield energy treatment exhibited the significant impact on the physical, thermal and spectral properties of SFRE-M. Based on this, it is presumed that Mr. Trivedi's biofield energy treatment can modulate the physicochemical properties of SFRE-M so that it could be utilized as a better medium for the cell culture of primary mammalian cell *in vitro*.

## Abbreviations

NCCAM: National Center for Complementary and Alternative Medicine;

NIH: National Institute of Health;

XRD: X-ray diffraction;

TGA: Thermogravimetric analysis;

DTG: Derivative Thermogravimetry

## Acknowledgements

The authors would like to thank the Trivedi Master Wellness, Trivedi Science, and Trivedi Testimonials for their sturdy support during this study. Authors would also like to acknowledge the whole team of MGV pharmacy college, Nashik for the instrumental facility, used in this work.

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