PVA/(2-PROPANOL) BASED BEE-HONEY(CORE) AND POVIDONE- IODINE (SHEATH) COAXIAL ELECTROSPUN BEADS ON STRING NANOFIBROUS SCAFFOLD FOR CONTROLLED DRUG RELEASE

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INTRODUCTION

Bee honey has shown promise to improve wound healing in both acute as well as chronic wounds. Many publications have been published supporting the potential of honey in preventing and removing infections on wounds. Honey contains anti-inflammatory, antiseptic, anti-microbial, and antibiotic effects.

Povidone-iodine is a broad-spectrum antiseptic used typically to treat and prevent wound infection. It can be used to treat small wounds, burns, abrasions, and blisters in first aid. Povidone-iodine has longer-lasting antiseptic effects than iodine tincture due to its sluggish absorption into soft tissue, making it the preferred choice for lengthier procedures.

In this particular study our main aim is to specifically prepare beads on string nanofibers by coaxial electrospin method by employing the transition phase of electrospraying and electrospinning which is done by increasing the polymer concentration and certain other parameters. This involvement of polymer concentration and other parameters has been stated in literature related to Maedeh Zamani et al(2013)[1] and Maria Nikolaou et al(2018)[2]. Another objective of this study is to explore the research gap on drug release profiles where one is bee honey and other is povidone iodine and respectively this is the first time where natural and synthetic drugs are employed together in coaxial electrospinning. Our preparation of beaded nanofibers relates to studies of Tingxiao Li et al(2016)[3],Tingxiao Li et al (2019)[4], Somvipart et al (2013)[5] and Ding Li et al (2021)[6] with respect to drug release profiles and behaviours.

Poly vinyl alcohol (PVA) is a hydrophilic polymer [7] with good chemical [8] and thermal stability [9] and has been proposed for various biomedical, biomaterial and Nano biotechnological applications [11], [12]owing to its good mechanical properties [13],[14], biocompatibility[15],[16], nontoxicity [17], [18], and biodegradability [19], [20].

Even though some consider beads on string nanofibers as something useless for some applications because of its non-uniformity, recently with the leap of nano-medicine and drug encapsulation some researchers have noticed unique features of beads-on-a-string structure [3] and discovered that the size of particles can be manipulated so as to control the drug releasing efficacy [4]. Considering the above stated literature, we were able to prepare beaded nanofibers by coaxial electrospinning core solution (polyvinyl alcohol based honey solution) and sheath solution (polyvinyl alcohol based povidone iodine solution). This is the first time in the world where electrospinning of a solution with a similar base as the core and sheath solution has been conducted. Preparation of these beaded nanofibers could be called as sheath solution induced transition from electrospraying to electrospinning of bee honey (Apidae family) core solution which is the opposite of the study of Luo et al (2014) which was about core liquid induced transition from coaxial electrospray to electrospinning of low-viscosity poly(lactide-co-glycolide)sheathsolution)[21].

METHODOLOGY

Preparation of coaxial electrospinning solutions

Table 1.1- Weighted proportions of PVA, Honey, Povidone iodine,2-Propanol and Distilled water in the preparation of core and sheath solutions.

	Core Solution				Sheath Solution			
Spin No:	PVA(g)	Honey(g)	2-Propanol(g)	Water(g)	PVA(g)	Povidone Iodine(g)	2-Propanol(g)	Water(g)
1)	3	0.75	23.125	23.125	3	0.75	23.125	23.125
2)	3	0.75	23.125	23.125	4	1	22.5	22.5
3)	3	0.75	23.125	23.125	5	1.25	21.875	21.875

A series of solutions were prepared inorder to proceed the electrospinning as given in the above table 1.1. We have used 2-Propanol and Distilled water in equal amounts as the medium to prepare the relevant core and sheath solutions here, and the core solution proportions are fixed in the series of three electrospins while changing the proportions of the sheath solutions so as to observe the outcomes accordingly.

Table 1.2- (W/W) % of Core and Sheath solutions

	Core			Sheath		
Electrospin Sample No:	PVA Concentration (W/W)%	Honey Concentration (W/W)%	Weight Percentage (W/W)%	PVA Concentration (W/W)%	Povidone Iodine Concentration (W/W)%	Weight Percentage (W/W)%
1	6	1.5	7.5	6	1.5	7.5
2	6	1.5	7.5	8	2	10
3	6	1.5	7.5	10	2.5	12.5

According to table 1.2 it clearly depicts the core solution PVA and honey concentrations were kept constant while the sheath solution was prepared as a series of increasing concentrations but the ratios of concentrations between the PVA and povidone iodine is kept constant at a ratio of 4:1. Magnetic stirring parameters of all core and sheath solutions were 600rpm, 1/2 Hr,60^0C.

Preparation for coaxial electrospinning

Table 1.3- Coaxial electrospinning parameters

Electrospin Sample No:	Spinning Voltage(kv)	Flow rates(ml/h) core and sheath	Separation(cm)	Drum speed(rpm)	Sliding speed(mm/s)	Needle size
1	17	0.5	9	502	30	21G/15G
2	17	0.3	9	502	30	21G/15G
3	17	0.1	9	502	30	21G/15G

The prepared core and sheath solutions were fed to the electrospinning system of spinneret of outer and inner needle gauge of 21 G and 15G at relevant flow rates accordingly with the table 3.4 using two standard syringes using a syringe pump (Tonglii tech). A voltage of 17 kV was applied between the collecting aluminum foil and tea bag attached to the drum 9 cm away from the spinneret tip. The electrospinning was conducted at room temperature for 4 hrs to gain sufficient smooth nanofiber membranes which were stored safely overnight.

Surface morphology of beaded nanofibrous mats

The morphology of the beaded nanofiber samples were studied by a scanning electron microscope (EVO LS15 ZEISS, SEM Lab UOP) with different magnifications. Fabricated fiber samples on Aluminium foils and tea filter bags were sputtered with a thin gold layer prior to SEM analysis using a PVD sputtering machine (Quorum). Based on these SEM image analysis the average diameter of fibers and beads was determined using imageJ software.

Fourier transform infrared spectroscopy

FTIR analysis was employed to identify the chemical bonds and structures of bee honey and povidone iodine in the fiber mats. FTIR spectra of bee honey, povidone iodine and mixture of both were obtained by using Nicolet iS50 FT-IR spectrometer.

In-vitro honey and povidone iodine release study

To study the release behaviour of bee honey and povidone iodine from drug loaded beaded nanofibers; three fiber samples (according to the series) fabricated on tea filter bags were cut into 2cm x 2cm samples. Then three series of test tubes were prepared each with 10 test tubes filled with 0.9% NaCl saline solution. Individual series relevant to a unique fabricated condition were separately studied for release by following this procedure.10 cut samples of 2 cm x 2 cm were submerged in all 10 test tubes simultaneously under stopwatch count.. For each 5 min the submerged sample in the test tube series was taken one by one in 5 min intervals. For example after 5 mins remove one cut sample submerged in one test tube and after another 5 mins next one so that you will get a series of test tubes corresponding to cumulative release of honey and povidone iodine in the range from 5-50 mins. Repeat this process for the other two series as well.

Optical absorption measurements

The amount of bee honey and povidone iodine in saline samples were determined by a UV-VIS spectrophotometer (UV-2450 SHIMADZU) at wavelengths of 282 nm and 207 nm corresponding to bee honey and povidone iodine. Released amounts of bee honey was according to the calibration curve of bee honey in saline prepared from solutions containing 0.05, 0.1, 0.15, 0.2, 0.25 mg/ml. Released amounts of povidone iodine was according to the calibration curve of povidone iodine in saline prepared from solutions containing 0.1, 0.2, 0.3, 0.4, 0.5 mg/ml.Comparison of the release behaviours of bee honey and povidone iodine at 3 different concentrations were statistically analyzed using Microsoft excel.

RESULTS AND DISCUSSION

Morphological characterization

Morphology of SEM images of the three fabricated samples according to all constant electrospinning parameters except the flow rate and increasing sheath concentration of PVA and Povidone iodine keeping core PVA and honey concentration constant are given below.

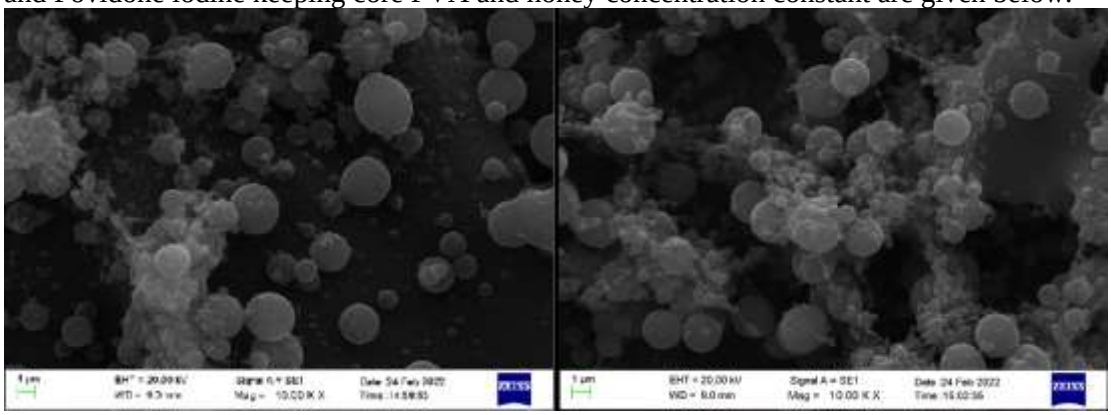


Fig 1.1 Sample 1-Sheath solution 7.5 W/W% - Flow rate 0.5 ml/h

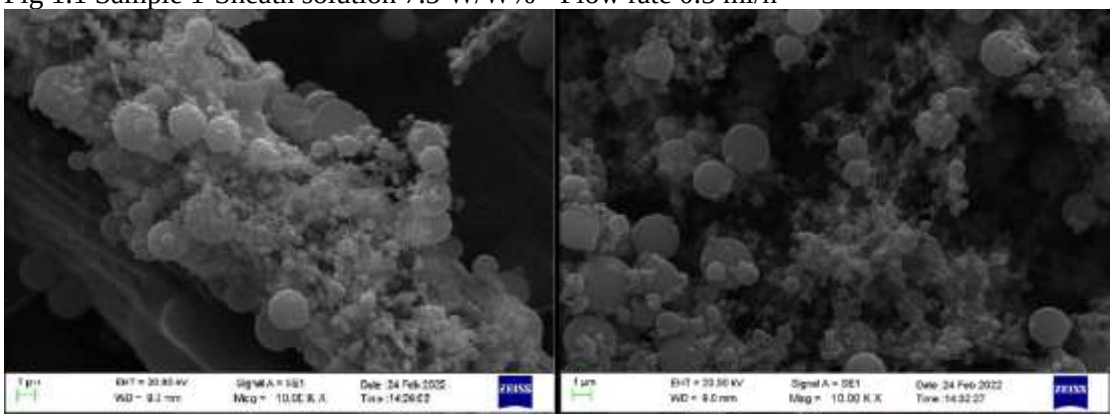


Fig 1.2 Sample 2-Sheath solution 10 W/W% - Flow rate 0.3 ml/h

As you can see from the Fig 1.1 sample 1 and Fig 1.2 sample 2 are more prominent with micro and nanoparticles where hints of potential formation of beaded nanofibers can be observed whereas Fig 1.3 sample 3 clearly shows the intended formation of beads on string nanofibers.

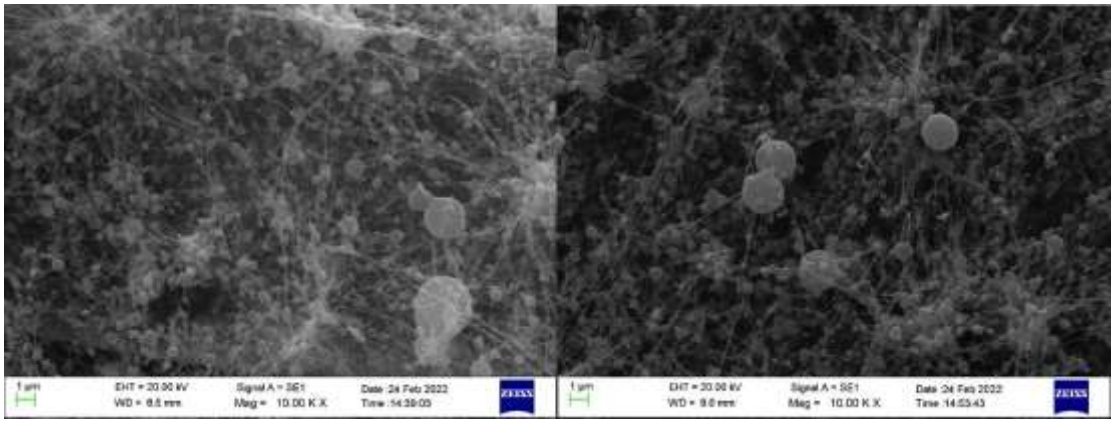


Fig 1.3 Sample 3-Sheath solution 12.5 W/W% - Flow rate 0.1 ml/h

Table 1.4- Morphology of SEM images and varying parameters of the samples.

Sample	Sheath solution (W/W%)	Core and sheath Flow rates(ml/h)	Morphology of SEM images
1	7.5	0.5	Micro and Nanoparticles
2	10	0.3	Micro and Nanoparticles
3	12.5	0.1	Beads on string nanofibers

Image J of software analysis shows that average particle count per μm^2 for Fig 1.1 sample 1 is 0.19513 while average particle count per μm^2 for Fig 1.2 sample 2 is 0.14407 which clearly portrays that average particle count per μm^2 of the sample has decreased with increasing sheath weight percentage (W/W%) and decreasing flow rates of sheath and core solutions.

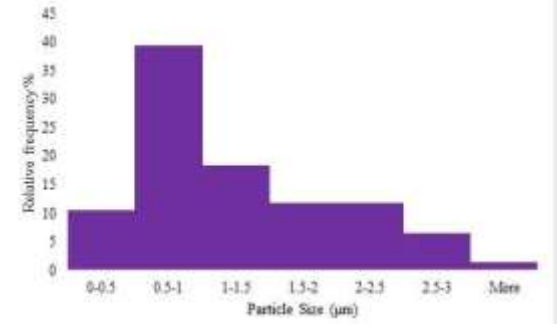


Fig 1.4 Sample 1 bar graph -Particle size

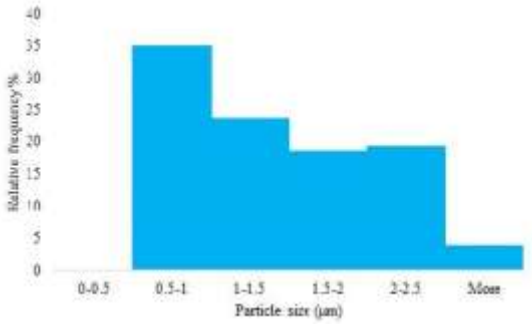


Fig 1.5 Sample 2 bar graph -Particle size

Fig. 1.4 and Fig 1.5 depicts the particle size trends of sample 1 and sample 2 with respect to increment in sheath weight percentage from 7.5 W/W% to 10 W/W% (concentration) and decrement of flow rates from 0.5 to 0.3 ml/h.

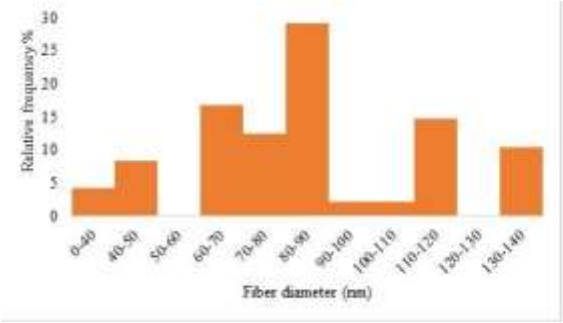


Fig 1.6 Sample 3 bar graph -Fiber diameter

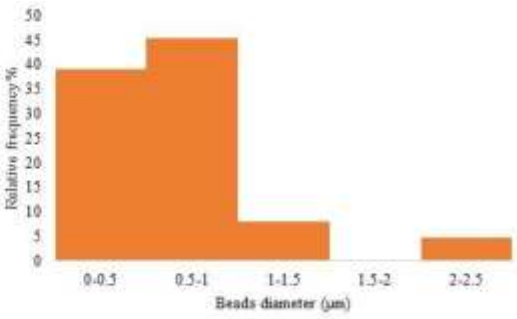


Fig 1.7 Sample 3 bar graph - beads diameter

From the Fig 1.3 sample 3 considering 100 beads on string nanofibers through image J software analysis as depicted by Fig 1.6 higher relative frequency percentage of nanofibers was observed in the range of 80 to 90 nm in fiber diameter. Higher relative frequency percentage of beads was observed in the range of 0.5 to 1 µm as given in Fig 1.7. Through the morphological analysis it clearly shows that we were able to transfer from coaxial electrospray to coaxial electrospin to produce beads on string nanofibers successfully using this approach.

FTIR Characterization

FTIR was used to verify the chemical constituents, composition and also to verify that there is no significant bond formation in between bee honey and povidone iodine when it is released to the wound. Using both bee honey and povidone iodine in wound treatments has been tested by Shukrimi A. et al (2008) on his comparative study between honey and povidone iodine as dressing solution for Wagner type II diabetic foot ulcers [22] and also by Jasmine Parimal et al. (2014) on her comparative study on the effect of honey and povidone iodine ointment on pain, wound healing and quality of life of patients with varicose ulcer [23]. These studies prove that there is no ill effect on the mixing of the two drugs. In accordance with the FTIR analysis we were able to prove that there is no significant effect on new bond formation due to mixing both components after slow release from the wound dressing. Fig 1.8 depicts stacked FTIR spectra of bee honey, povidone iodine and bee honey + povidone iodine. Characteristic peaks of povidone iodine (PVI), bee honey are given in the following table 1.5 and table 1.6 respectively.

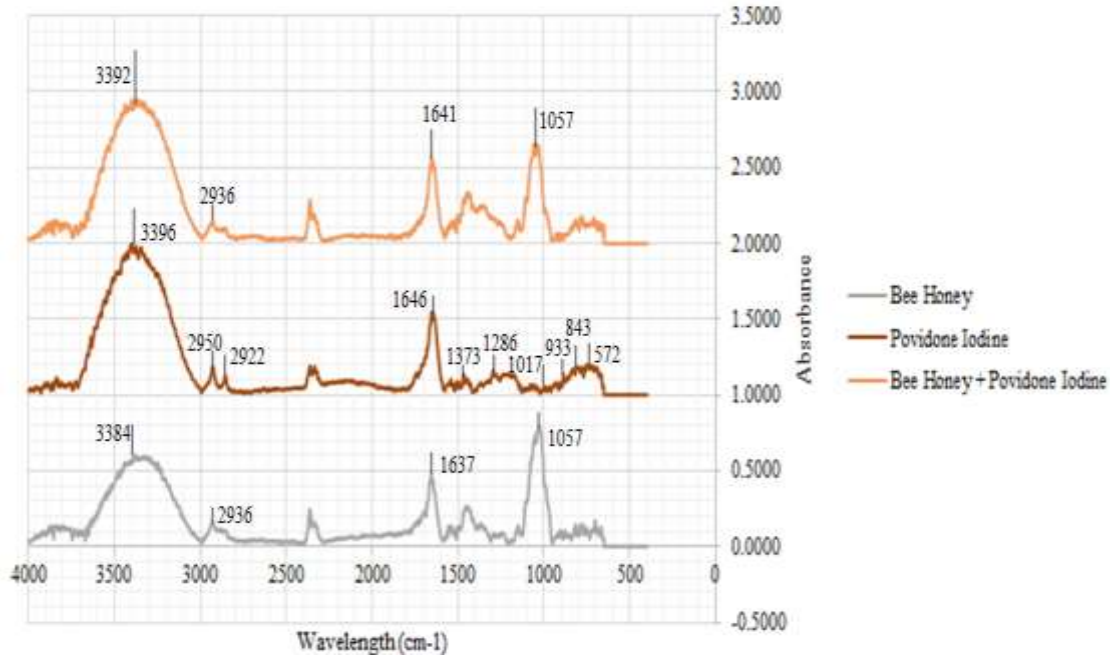
Table 1.5 Characteristic peaks of PVI

Pure PVP (cm^{-1})	Vibrational mode
3396	ν(O-H)
2950	Asymmetric ν(CH2) of pyrrole ring
2922	Symmetric ν(CH2) of chain
1646	ν(C=O)
1373	δ(C-H)
1286	CH2 wagging ν(C-N)
1017	C-C,CH2 rock
933	C-C bond
843	δ(CH2)
572	δ(N-C=O)

Table 1.6 Characteristic peaks of bee honey

Bee honey (cm-1)	Vibrational Mode
3384	O-H
2936	C-H
1637	C=O
1057	C-O

Fig 1.8 Stacked FT-IR Spectra



In vitro Drug Release

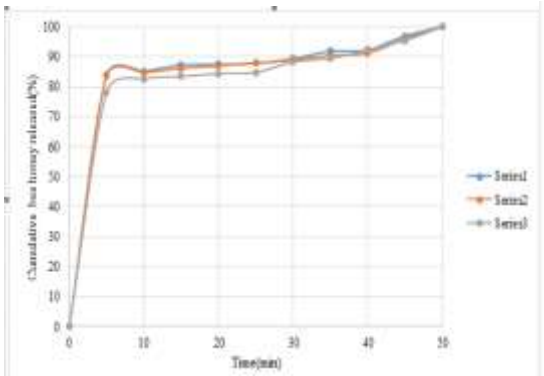


Fig 1.9 Release profile of bee honey

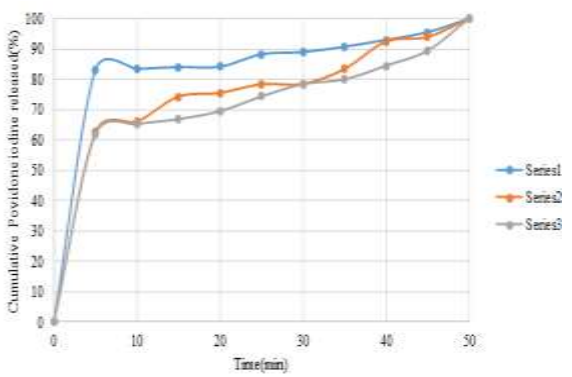


Fig 2.0 Release profile of povidone iodine

The cumulative release of bee honey and povidone iodine from electrospun particle and fiber weds fabricated on tea filtering bags as a function of the immersed time interval is shown in figures 1.9 and 2.0. Here, series 1 refers to the sample 1 where weight percentage of sheath solution was 7.5 W/W%, series 2 refers to the sample where weight percentage of sheath solution was 10 W/W% while series 3 refers to the sample where weight percentage of sheath solution was 12.5 W/W% as given in tables 1.1 and 1.2. From figure 1.9 it could be observed that the release profiles of bee honey from three samples of increasing sheath solution concentrations and decreasing flow rates are very much similar even though first two samples consist of particles more than beaded fibers where as prominent beaded fibers were seen in sample 3 which is the reason for the release profile of series 3 is having slower release compared to micro and nanoparticles.Which interprets the embedded nature of bee honey inside beaded nanofibers due to coaxial electrospinning. Initial release at short time after immersion can be seen due to swelling of the beaded nanofibers. On the other hand from figure 2.0 it could be observed that the release profiles of povidone iodine of three samples. First series having more particle density and less fibers show higher release of povidone iodine with an initial release whereas series 2 with lower particle density and more fibers comparatively shows slower release compared to sample 1.Third series has the slowest release rate of povidone iodine and shows bits of similarities in release compared to second series.

Table 1.7-ANOVA results of the release behaviour of bee honey

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	28.5424	2	14.2712	0.44372	0.64624	3.35413
Within Groups	868.391	27	32.1626			
Total	896.934	29				

To understand the release behaviour of bee honey let's consider the statistical analysis given in the above table 1.7.The anova analysis gives a p value greater than 0.05 ($p>0.05$) which means honey doesn't have a substantial effect on the release behaviour. Hence bee honey can be used as a kind of common antibiotic besides drugs to improve the wound healing efficiency. The behavioural analysis of bee honey in fact corresponds and correlates with the study by maleki et al (2012) [24].

Table 1.8-ANOVA results of the release behaviour of bee honey

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	867.602	2	433.801	5.9007	0.00824	3.40283
Within Groups	1764.41	24	73.5169			
Total	2632.01	26				

To understand the release behaviour of povidone iodine let's consider the statistical analysis given in the above table 1.8.The anova analysis gives a p value less than 0.05 ($p<0.05$) which means the it's in the statistically significant level and definitely has an effect on release behaviour of PoI.

CONCLUSIONS/RECOMMENDATIONS

According to Ayurveda and siddha medical writings, bee honey was the first wound dressing and it has been utilized for wound healing and care because of its medicinal value. Povidone iodine has been a significant part of modern day common drugs used for wound healing. In this study, a novel PVA/- Propanol based bee honey core and povidone iodine sheath coaxial electrospun beads on string nanofibers were successfully fabricated in sample 3 using a unique approach in the transition phase of coaxial electrospraying to coaxial electrospinning. Particle count and fiber diameters were controlled through sheath solution weight percentage

and flow rates. This unique approach of electrospinning kept all the parameters constant except the flow rates. This is the first time it has been tested to use the same based medium PVA as both core and sheath solutions to mix the relevant drug. When the sheath concentration increased, beads on string fibers were more prominent and resulted in a slower rate of povidone iodine while bee honey release rate didn't have significant change in release rates. Even though beads on string nanofibers are not considered much in applications this study shows that it has better application potential in the field of slow drug release. Where in this case slow drug release of povidone iodine and the natural antibiotic care of bee honey is an interesting combination for wound dressing applications and many types of wounds. Further adjusting and improving this study could result in coaxial nanofibers which will be studied in later experiments for drug release applications.

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