https://doi.org/10.48047/AFJBS.6.14.2024.7256-7284

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

PVP-I LOADED COLLAGEN DUSTING POWDER BY *IN-VITRO* AND *IN-VIVO* WOUND HEALING ACTIVITY

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Volume 6, Issue 14, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.7256-7284

ABSTRACT:

Wound healing is complex phenomenon which involves various cell types, growth factors, cytokines and vascular system. The dusting powder was chosen as an ideal formulation for the open wounds as it absorbs the excessive moisture which prevents the microbial growth. Povidone iodine as antiseptic, collagen as base and excipients are incorporated in the formulated dusting powder using QBD principles. Analytical techniques such as FTIR Spectroscopy, DSC were employed to check for the interaction among the ingredients used. The drug release kinetics follows higuchi model. From the standardisation and assay the concentration of Povidone iodine in dusting powder was found to be 0.025mg/ml. The Dose of 100µg/ml was found to show effective multiplication of cells in *in-vitro* proliferation assay using Human Dermal Fibroblast cell line. Adult zebra fish were used in the study as they mimic 70% of human gene. The dusting powder was applied on the amputated caudal fin of the fish to determine the regenerative capacity. The sagittal section of the fin of each group was taken for the histological examination. The study revealed that the formulated dusting powder was found to accelerate the healing of wound and prevent the microbial infection.

Keywords: Differential Scanning Calorimetry (DSC), Fourier-Transform Infrared Spectroscopy (FTIR), Quality by design (QBD), Wound healing.

1. INTRODUCTION

Wound are an unpreventable and ineluctable event in life. Wound healing is a dynamic and complex repair process which begins after an injury. The wound can be defined as the damage of cellular and anatomically elongated tissues in the body which disturb the normal alignment of the cells [1]. Skin is the organ of protection, regulation, sensation and protect against UV light. It comprised of collagen fibres, elastic tissue, fibroblast, histiocytes [2].

Phases of wound healing are haemostasis, inflammation, proliferation, remodelling, and scar formations [3]. The clot and platelets around the wound release cytokine and growth factors such as Platelet Derived Growth Factor (PDGF), Transforming Growth Factor-β(TGF-β), Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), Vascular Endothelial Growth Factors (VEGF), Interleukin-1(IL-1), are pro-inflammatory cytokine which activates the neutrophils, macrophages and fibroblast [4]. In hemostasis platelets produce thrombin for the development of the clot. Migration of the neutrophils and other components followed by the inflammatory stage which is the second stage of repair mechanism.

At the initial stages the growth factors produced by the neutrophils and macrophages helps in eradicating the bacteria and remove dead cells and foreign bodies present in the wound area by phagocytosis. Angiogenesis plays a crucial role in the inflammatory phase and proliferative phase of wound healing [5]. After the migration of the fibroblast, the collagen genesis occurs. Collagen helps in the proliferation of the cells and tissue. Keratinocyte growth factor (KGF-1and KGF-2) are synthesized by the fibroblast which stimulate the other keratinocytes to reach the site of wound, proliferate the cells [4]. In re-epithelialization a thin layer of the epithelial cells was formed. The remodeling is characterized by the contraction of wound and remodeling of the collagen. The final outcome of the repair process is formation of the scar [3].

In order to increase the patient compliance by cut down pain, discomfort and scarring PVP-I loaded collagen-based dusting powder was spotted as wound care management.

Homogenous dusting powder has easy flow, spread uniformly and stick to the skin when applied [6]. So, it is chosen over other formulation as it mixes with the fluid of the wound and it increases the swiftness of open wound. Dusting powder assuages inflammation and microbial growth by drying and cooling the skin. It increases the surface of evaporation and heat exchange by hastening the sebum and hidrosis [7]. Collagen is the major insoluble fibrous material found in the extracellular matrix of the skin in conjugate with elastin and hyaluronic acid [8]. It has become an inevitable source in treating tissue repair and in the regeneration of tissues. Type-1 collagen is selected for dusting powder formulation as it has potent re-epithelization property. The increase in the production of collagen due to the activation of fibroblast which is present in connective tissue and it is sensitive to physical and chemical stimuli. The collagen has the purpose to aid in the tissue resist stretching [9]. Povidone Iodine contains an anti-bacterial property that is effective against gram-positive and gram-negative microorganisms and used as an antiseptic agent for wound infection [10]. It acts as the reservoir of iodine and penetrates povidone-iodine into the skin layer to exhibits its broad-spectrum activity. It exhibits antimicrobial effect by oxidising the cell wall of pathogen and deactivates DNA/RNA and protein [11].

Chitosan is a polycationic linear polysaccharide which interacts with the cell surface and forms an impermeable layer to prevent the transport of solutes [12]. In wound healing, the chitosan helps in coagulating the blood by activating the regeneration of the thrombin [13]. In dusting powder, talc is used as a glidant, skin protective, and adsorbent [14]. Recent trends following QbD (Quality by design) is the most appropriate approach for every aspect of formulation development to emphasize product and process understanding and process control on. In the current study QbD methodology was used to examine the effects of testing parameters and formulation variables on the prepared dusting powder in the view to ensure quality and mitigate risk, saving formulation time and cost [A]. The wound healing potential of PVP-I loaded collagen-based dusting powder was determined using *in-vitro* proliferation assay. For this, HDF (human dermal fibroblast) cell line was utilized in this study [15].

Zebrafish has fast external development, short generation time, so it is used in wound healing study. The zebrafish has 70% homology with human genes and shares anatomic and physiological characteristics [16]. Hence in this study the efficacy of the prepared formulation was tested using the caudal fin which was amputated and regeneration was monitored on specific days based on the literature, Akimenko *et al.,* 2003 [17].

2. MATERIALS AND METHODS

2.1. Materials

Povidone Iodine I.P was gifted by ADANI PHARMACHEM PVT LTD and Collagen was also gifted by SYNERHEAL PVT LTD. Chitosan and Talc were purchased from MERCK. All other chemicals used were of analytical grade.

2.2. Design and formulation of dusting powder

The master formula used for the preparation of dusting powder was taken from British Pharmacopoeia. 63% of Collagen, 5% of Povidone Iodine I.P, 30% Chitosan and 2% Talc was weighed in electronic balance and mixed well using a mortar & pestle. The powder was stored in an amber-coloured glass jar. The Povidone Iodine loaded with Collagen along with base was formulated by trituration method. The formulation was optimized using the Quality by Design – Design expert software and the ANOVA was calculated for the Angle of repose and water holding capacity using the Response surface quadratic model (table 1).

Table 1: Experimental run of the dusting powder formulation

Run	Collagen	Povidone Iodine	Chitosan	Talc	Angle of repose	Water Holding Capacity
1	315	25	150	10	33.2	1690
2	315	25	200	15	28.3	1680
3	230	25	100	10	27.5	1678
4	315	30	150	5	29.7	1682
5	400	20	150	10	26.8	1673
6	315	30	200	10	30.3	1669

7	230	25	200	10	26.9	1672
8	315	30	100	10	25.4	1679
9	400	25	100	10	29.8	1675
10	315	20	150	15	30.6	1659
11	315	30	150	15	26.2	1664
12	400	25	150	15	23.8	1674
13	230	25	150	5	24.7	1659
14	230	20	150	10	23.9	1653
15	230	30	150	10	27.9	1663
16	315	25	150	10	33.2	1690
17	315	25	150	10	33.2	1690
18	315	25	100	5	30.6	1648
19	400	25	150	5	27.9	1654
20	230	25	150	15	29.2	1669
21	315	25	100	15	24.8	1670
22	315	25	150	10	33.2	1690
23	315	25	200	5	26.3	1655
24	400	30	150	10	29.4	1664
25	315	20	200	10	31.3	1672
26	315	25	150	10	33.2	1690
27	315	20	150	5	30.1	1626
28	400	25	200	10	30.4	1667
29	315	20	100	10	29.3	1676

2.3 characterization of PVP-I loaded collagen dusting powder

Interventionary studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.

2.3.1. ANGLE OF REPOSE

To estimate the flow properties of dusting powder, the hollow cylinder method was used [18]. The formulation is placed in the hollow cylinder and allowed to flow at a particular velocity which forms a conical heap [19]. The static angle of repose was calculated from the height (h) and diameter of the pile as per arctan rule:

$$\Theta = \tan^{-1} h/r$$

where, h = height,

r = radius

2.3.2. WATER HOLDING CAPACITY

The water holding capacity of dusting powder was determined partly by their capacity for absorbing or retaining excessive fluid on the wound which enhances healing environment. **[20].** The water holding capacity was calculated at the various time intervals.

$$\text{Water holding capacity} = \frac{W_2 - W_1}{W_1}$$

2.3.3. FOURIER-TRANSFORM INFRARED SPECTROSCOPY

FTIR spectrums of collagen, povidone iodine I.P, base and formulation of combined drugs were evaluated. This was analysed to prove the significant changes happened (if any) in

the combination because of the interaction existing between povidone and other substance present in the formulation. They can be shown in the spectrum by significant shifts in their peaks **[21]**.

2.3.4. DIFFERENTIAL SCANNING CALORIMETRY

DSC is a widely accepted technique to measure the denaturation temperature (Td) of protein, and it gives a better understanding about the folding/unfolding of a protein under the influence of temperature. Thermo scans from 20°C to 300°C were performed under inert nitrogen atmosphere at a ramp speed of 10°C/min to determine the denaturation temperature of samples (Formulated dusting powder, Collagen, Povidone Iodine, Base) **[22]**.

2.3.5. STANDARDISATION AND ASSAY OF PVP-I:

Povidone Iodine I.P was dissolved in 0.05M acetic acid solution(1mg/ml). Stock solution (100µg/ml) was prepared from the above solution and made up with phosphate buffer (pH7.4). Serial dilution of various concentrations was prepared by pipetting from the stock solution. Absorbance was measured in UV spectrophotometer at 240nm.

Formulation was dissolved in 0.05M acetic acid(1mg/ml) and it was set for overnight. The concentration of 10µg/ml was prepared using phosphate buffer(7.4pH) from the above solution. Absorbance was measured in UV spectrophotometer at 240nm and interpolated **[23]**.

2.3.6. *IN-VITRO* KINETICS RELEASE

The *in-vitro* release kinetics was carried out using dialysis membrane technique (DM). The dusting powder was packed in a dialysis bag containing phosphate buffer (7.4pH) that is subsequently sealed and placed in phosphate buffer solution. The diffused drug in the outer compartment was sampled for analysis at various intervals. Absorbance was measured at 240nm (D'Souza *et al.*, 2014).

2.4. *IN-VITRO* cell line assay

Cell proliferation of *PVP-I loaded collagen-based dusting powder* on Human dermal fibroblast (HDF) was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay **[24]**. Human dermal fibroblast (HDF) cells were seeded in a 96 well plate and incubated at 5 % CO_2 at 37°C. After overnight incubation, the culture medium was replaced with fresh medium of DMEM containing 0.5% FBS. Formulated dusting powder at various concentrations was added to each well. The cells were also treated with the standard 10 % Fetal Bovine Serum (FBS). At the end of incubation, 100 µl MTT (5 mg / ml stock) was added and incubated for 3 hours at 37°C. The purple-coloured formazan crystals formed by the reduction of MTT in presence of mitochondrial dehydrogenase enzyme. The crystals were dissolved in 100 µl of DMSO by shaking for 15 min at room temperature. The intensity of the colour developed was absorbed at 570 nm in a 96 well plate. The percentage of cell viability was calculated

$$\% \text{ Growth Viability} = \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

Percentage of cell viability was plotted against concentrations of test samples. Three sets of experiments were performed in triplicate in and the data were presented as mean ± SD.

2.5.IN-VIVO WOUND HEALING ACTIVITY USING ZEBRA FISH MODEL

The Zebra fish has been regarded as powerful model for the regeneration studies as it has rapid migration and rearrangement of the epithelial cells.

The fishes were divided into 5 groups (n=40) (table 2)

Based on the effective dose found by the *in-vitro* cell line study, various groups of samples were separately mixed with petroleum jelly of concentration(100µg/ml) by geometric dilution. The wound healing activity in zebra fish was studied by inducing wound by caudal fin amputation method. In this study the full fin amputation method was followed. The zebra fish was anesthetized using 0.1% tricaine. In the caudal fin a single cut was made 5mm from the posterior end of the fin **[25]**. Sufficient amount of the sample was applied to every group of fish. Some of the parameters such as temperature (28° C), dissolved oxygen (D.O), pH, estimation of chlorine in water are monitored. The growth

was measured on day 0, day 5, day 7. The images were captured using USB-camera. The growth of the fin is measure using the software Image J.

Table 2: Grouping of zebra fish

GROUP I (C)	**CONTROL**
GROUP II (C+B)	COLLAGEN + BASE (CHITOSAN+ TALC)
GROUP III(P+B)	POVIDONE IODINE + BASE (CHITOSAN + TALC)
GROUP IV (C+P)	FORMULATION (POVIDONE IODINE +COLLAGEN + CHITOSAN+TALC)
GROUP V (B)	BASE (CHITOSAN& TALC)

2.6 HISTOPATHOLOGICAL EXAMINATION

Hematoxylin and eosin (H&E) staining is essential for the identification of various blood cells and tissues **[26]**. The fishes were euthanized and transverse cut was made to get the full caudal fin on 7^{th} day and preserved in formalin. The sagittal section of the fin from each group was taken using the microtomb. followed by staining. The slides were examined for red blood cells, white blood cells (neutrophils, eosinophils, basophils) and thrombocytes **[27]**.

3. RESULTS

3.1. ANGLE OF REPOSE

The RSM was used to identify the optimum area of interaction and to determine the interactive effects (Table 3) (Figure 1). The RSM for the response angle of repose with the variables A, B, C, D was statistically analysed and the model was found to be significant with $p < 0.05$. The height of the powder forming the cone, h and the radius, r of the base was measured. The angle of repose (θ) was calculated from $\Theta = \tan^{-1} h/r$ for collagen, povidone iodine, base and formulated dusting powder (Table 4) **[28]**. The following table represents the type of flow:

Table 3: Angle of repose

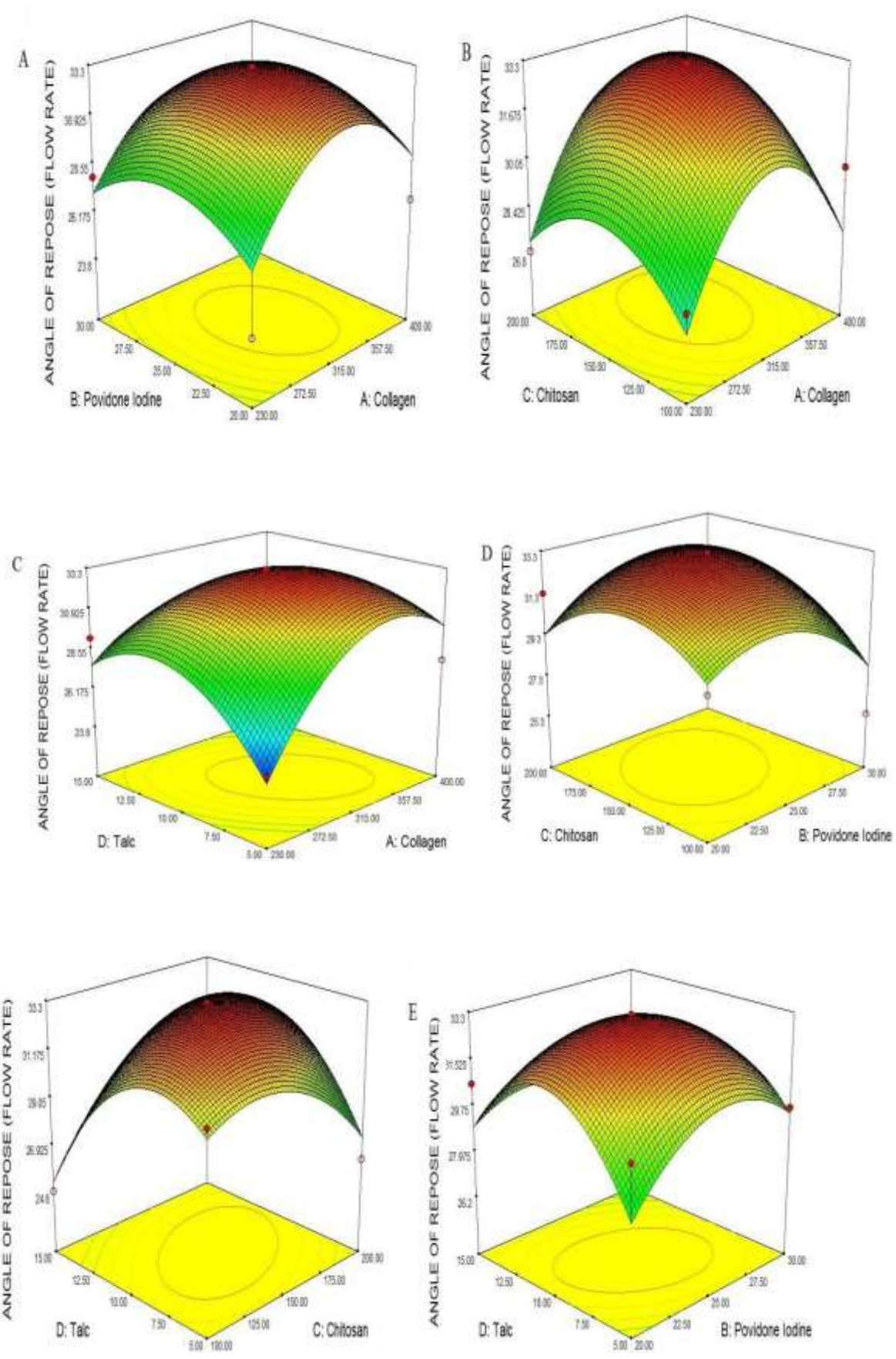
Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	182.1214	14	13.00867	3.377361	0.0148	Significant
A-Collagen	5.333333	1	5.333333	1.384661	0.2589	
B-Povidone Iodine	0.800833	1	0.800833	0.207915	0.6554	
C-Chitosan	3.100833	1	3.100833	0.80505	0.3848	
D-Talc	3.413333	1	3.413333	0.886183	0.3625	
AB	0.49	1	0.49	0.127216	0.7267	
AC	0.36	1	0.36	0.093465	0.7643	

AD	18.49	1	18.49	4.800445	0.0459	
BC	2.1025	1	2.1025	0.545859	0.4722	
BD	4	1	4	1.038495	0.3255	
CD	15.21	1	15.21	3.948879	0.0668	
A^2	81.17128	1	81.17128	21.074	0.0004	
B^2	24.66486	1	24.66486	6.403587	0.0240	
C^2	24.66486	1	24.66486	6.403587	0.0240	
D^2	59.84696	1	59.84696	15.5377	0.0015	
Residual	53.92417	14	3.851726			
Lack of Fit	53.92417	10	5.392417			
Pure Error	0	4	0			
Cor Total	236.0455	28				

Table 4: Measurement of Angle of repose

SAMPLE	**ANGLE OF REPOSE**	**TYPE OF FLOW**
FORMULATION	26.53°±2.78	EXCELLENT
COLLAGEN	33.20°±2.37	GOOD
POVIDONE IODINE	9.696°±1.31	EXCELLENT
BASE	16.61°±0.52	EXCELLENT

Figure 1: Response surface plot for angle of repose (flow rate)


A
ANGLE OF REPOSE (FLOW RATE)
B: Povidone Iodine
A: Collagen
B
ANGLE OF REPOSE (FLOW RATE)
C: Chitosan
A: Collagen
C
ANGLE OF REPOSE (FLOW RATE)
D: Talc
A: Collagen
D
ANGLE OF REPOSE (FLOW RATE)
C: Chitosan
B: Povidone Iodine
F
ANGLE OF REPOSE (FLOW RATE)
D: Talc
C: Chitosan
E
ANGLE OF REPOSE (FLOW RATE)
D: Talc
B: Povidone Iodine

A- Povidone iodine Vs Collagen; **B** – Chitosan Vs Collagen; **C** – Talc vs Collagen; **D** – Chitosan Vs Povidone Iodine; **E** – Talc Vs Povidone Iodine; **F** – Talc Vs chitosan

3.2. WATER HOLDING CAPACITY:

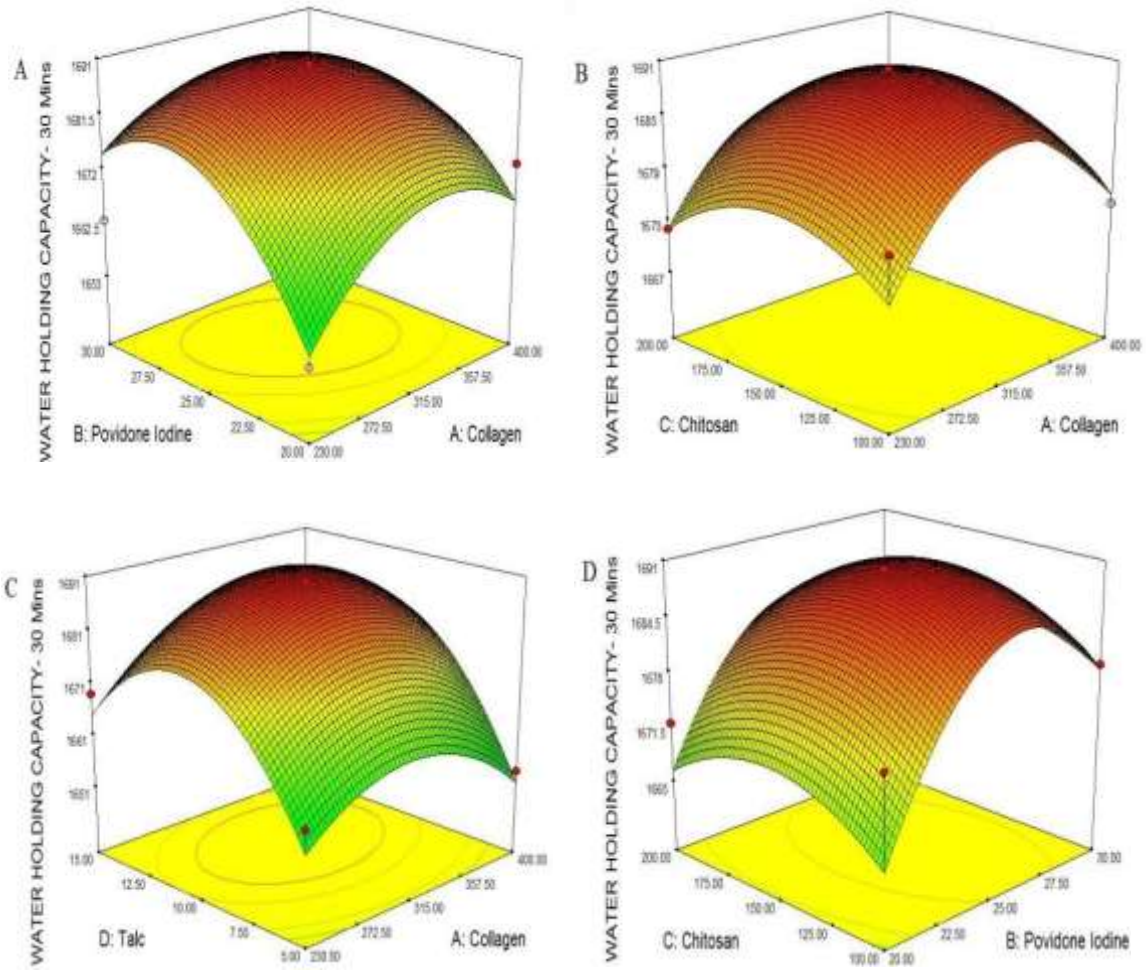
Water holding capacity reflects the water absorbing property of formulated dusting powder. The significance of the RSM model were studied (table 5) (Figure 2). The water holding rate of formulated dusting powder increased with time initially and it decreases after the equilibrium time. This may be due to cross linking structure of collagen dusting powder **[29]**. The formulation was found to carry good water holding capacity at the period of 30mins. The cross-linking degree and time, enhances the wound healing by absorbing the microbial rich water oozing out from the wound (Figure 3).

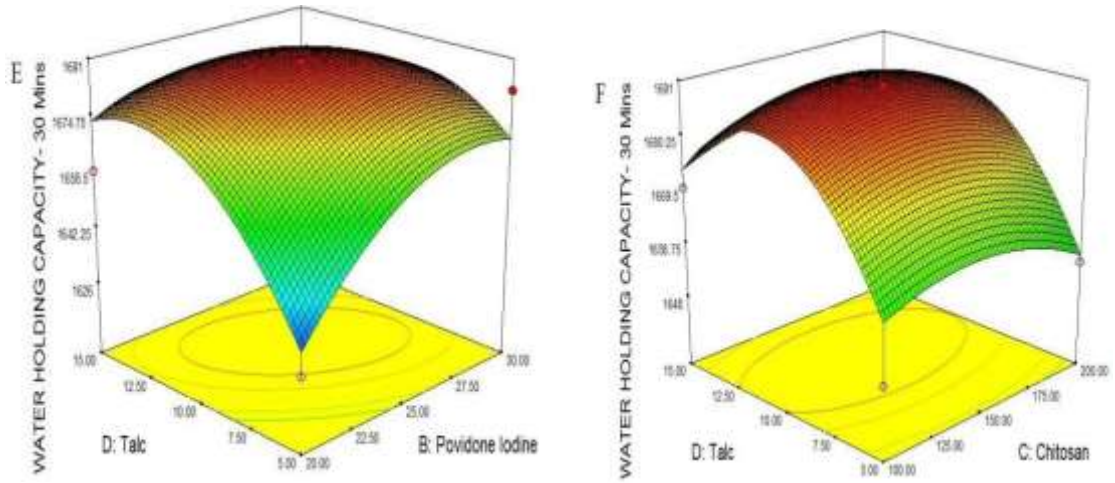
Table 5: Water holding capacity

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	4901.049	14	350.0749	4.276968	0.0051	significant
A-Collagen	14.08333	1	14.08333	0.17206	0.6846	
B-Povidone Iodine	320.3333	1	320.3333	3.913606	0.0679	
C-Chitosan	10.08333	1	10.08333	0.123191	0.7308	
D-Talc	705.3333	1	705.3333	8.617264	0.0109	
AB	90.25	1	90.25	1.102611	0.3115	
AC	1	1	1	0.012217	0.9136	
AD	25	1	25	0.305432	0.5892	
BC	9	1	9	0.109956	0.7451	
BD	650.25	1	650.25	7.944295	0.0137	
CD	2.25	1	2.25	0.027489	0.8707	
A2	749.5946	1	749.5946	9.158017	0.0091	
B2	1160.372	1	1160.372	14.1766	0.0021	

C2	214.4595	1	214.4595	2.620114	0.1278	
D2	2190.101	1	2190.101	26.75711	0.0001	
Residual	1145.917	14	81.85119			
Lack of Fit	1145.917	10	114.5917			
Pure Error	0	4	0			
Cor Total	6046.966	28				

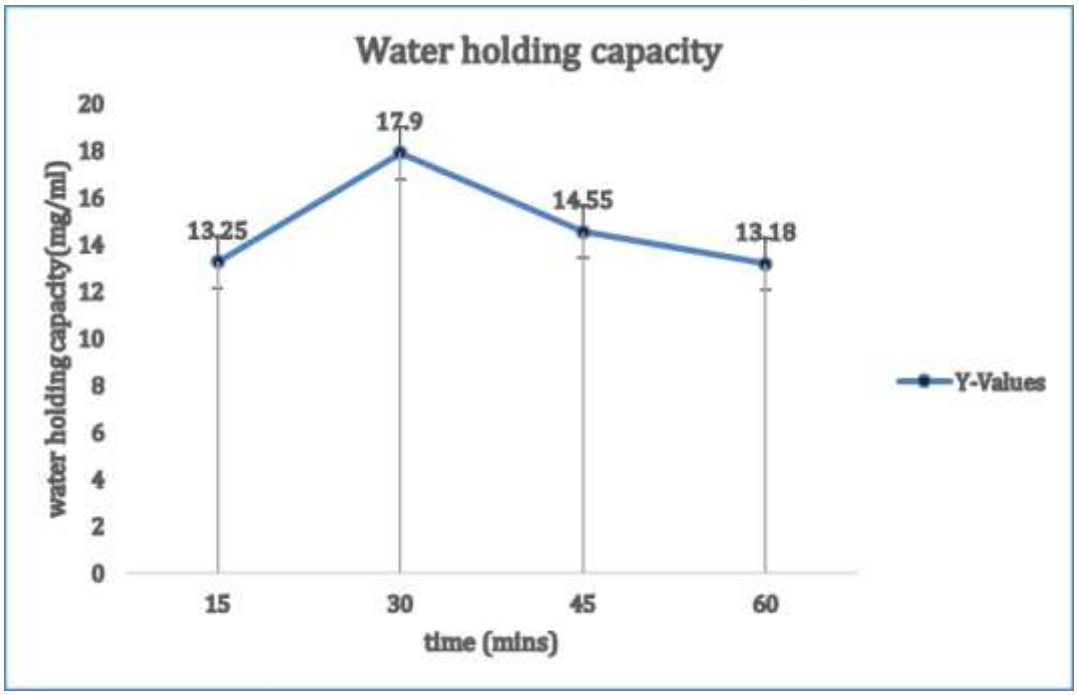
Figure 2: Response surface plot for water holding capacity (30 mins)





A- Povidone iodine Vs Collagen; **B –** Chitosan Vs Collagen; **C –** Talc vs Collagen; **D –** Chitosan Vs Povidone Iodine; **E –** Talc Vs Povidone Iodine; **F –** Talc Vs chitosan

Figure 3: Water holding capacity

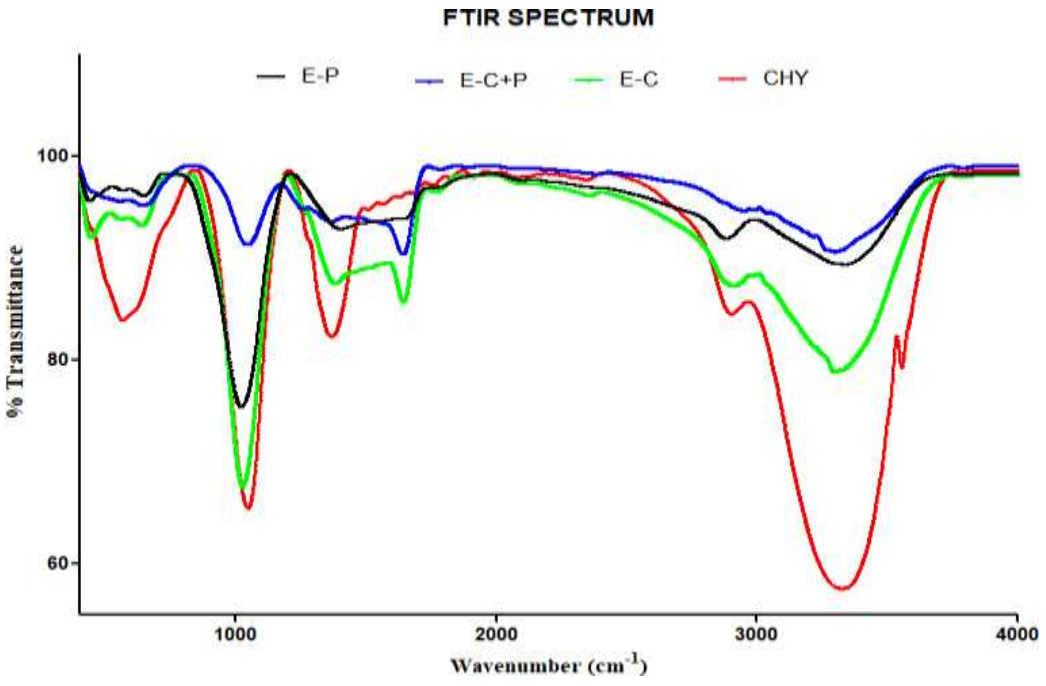


3.3. FOURIER-TRANSFORM INFRA RED SPECTROSCOPY:

FTIR spectroscopic analysis used to confirm the functional group and structural quality of the compounds present in the formulated dusting powder(Figure 4). The compounds combined in the dusting powder doesn't shows intrusion in the functional group therefore there is no significant change in active constituent **[21]**.

IR of the collagen dusting powder exhibited characteristic absorption bands in the regions of 1235-1250 cm^{-1} for amide III (C-N stretching) is at 1248 cm^{-1}. The absorption band at 1535 – 1555 cm^{-1} for amide II (N-H bending) was equivalent 1540 cm^{-1} in our IR band. The absorption band 1645-1658 cm^{-1} for amide I (C=O stretching) was at 1655 cm^{-1}. There is peak at 1658 which denotes alpha like helix. for 1679 cm^{-1} and 1626 cm^{-1} it shows bands for beta sheets. it shows peak for beta turns at 1691 cm^{-1} and 1669 cm^{-1}. The C–O–C shows a strong band at 1079 cm-1having range between 1150-1085 cm^{-1} .IR of collagen showed the absorption band at 1535 – 1555 cm^{-1} for amide II (N-H bending) was equivalent 1540 cm^{-1} in our IR band. It also showed absorption bands in the regions of 1235-1250 cm^{-1} for amide III (C-N stretching) is at 1248 cm^{-1}. The C–O–C shows a strong band at 1079 cm-1having range between 1150-1085 cm^{-1}.

Figure 4: FTIR Spectrum



E-P

E-P - FTIR of Povidone Iodine, **E-C+P -** FTIR of formulated dusting powder, **E-C** – FTIR of Collagen, **CHY-** FTIR of base (Chitosan & Talc)

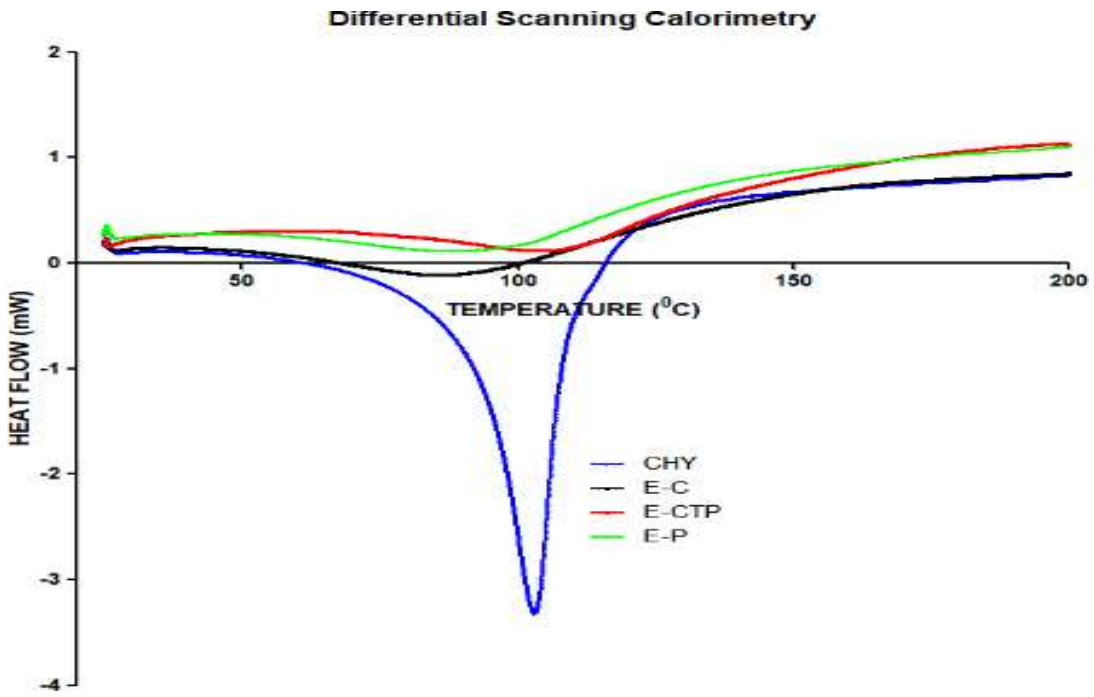
IR of povidone iodine has C=O stretching band was between 1700-1500 cm^{-1} and it showed 1700 cm^{-1} in our IR spectra. C=O Stretching (1685-1666), povidone showed at C=O conjugated ketone at 1662 cm–1(strong) and povidone iodine complex showed C=O conjugated ketone at 1659 cm^{-1} (strong).C-N stretching secondary amine ranges between 1190-1130 cm^{-1}, povidone showed the peak at 1171 cm^{-1} and for povidone iodine complex showed peak at 1163 cm^{-1}. Povidone has CH_2=CH has peak at 930 cm^{-1} (medium) and for povidone iodine complex at 931 cm^{-1} (weak), having normal range at 905-985 cm^{-1}.

In IR of base containing chitosan and talc, we can observe the infrared spectrum of chitosan. A strong band in the region 3291–361 cm^{-1} corresponds to N-H and O-H stretching, as well as the intramolecular hydrogen bonds. The absorption bands at around 2921 and 2877 cm^{-1} can be attributed to C-H symmetric and asymmetric stretching, respectively. These bands are characteristics typical of polysaccharide and are found in other polysaccharide spectra, such as xylan glucans **[30]** and carrageenan's **[31]**. The presence of residual N-acetyl groups was confirmed by the bands at around 1645 cm^{-1} (C=O stretching of amide I) and 1325 cm^{-1} (C-N stretching of amide III), respectively. We did not find the small band at 1550 cm^{-1} that corresponds to N-H bending of amide II. This is the third band characteristic of typical N-acetyl groups, and it was probably overlapped by other bands. A band at 1589 cm^{-1} corresponds to the N-H bending of the primary amine **[32]** The CH_2 bending and CH_3 symmetrical deformations were confirmed by the presence of bands at around 1423 and 1375 cm^{-1}, respectively. The absorption band at 1153 cm^{-1} can be attributed to asymmetric stretching of the C-O-C bridge. The bands at 1066 and 1028 cm^{-1} correspond to C-O stretching. All bands are found in the spectra of samples of chitosan reported by others **[33]**.

3.4. DIFFERENTIAL SCANNING CALORIMETRY

The DSC thermograms of native collagen, povidone Iodine, Chitosan, talc and collagen dusting powder was analysed (Figure 5). Thermal stability of collagen increased with the interaction of povidone Iodine indicated by formation of hydrogen bond interaction. The denaturation temperature of the formulated dusting powder was about 103.6°C, which was higher than that of native collagen was 86.5°C, PVP was 88.5°C and Chitosan and talc (powder base) was 109.2°C (Sionkowska *et al.,* 1999).

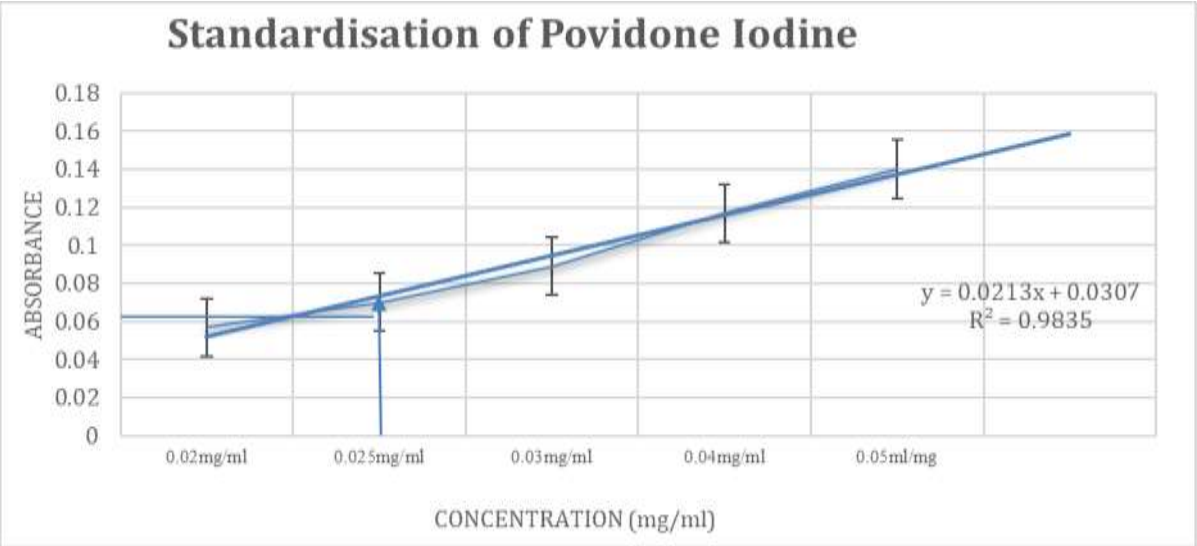
Figure 5: DSC Thermogram of native collagen, povidone iodine, chitosan, talc, and collagen dusting powder



3.5. STANDARISATION AND ASSAY

Absorbance was calculated at 240 nm for Povidone Iodine I.P. graph was plotted (Figure 6). The amount of Povidone iodine present in the formulated dusting powder was calculated from the standardisation curve. The concentration of PVP-I in formulated dusting powder was found to be 0.025mg/ml.

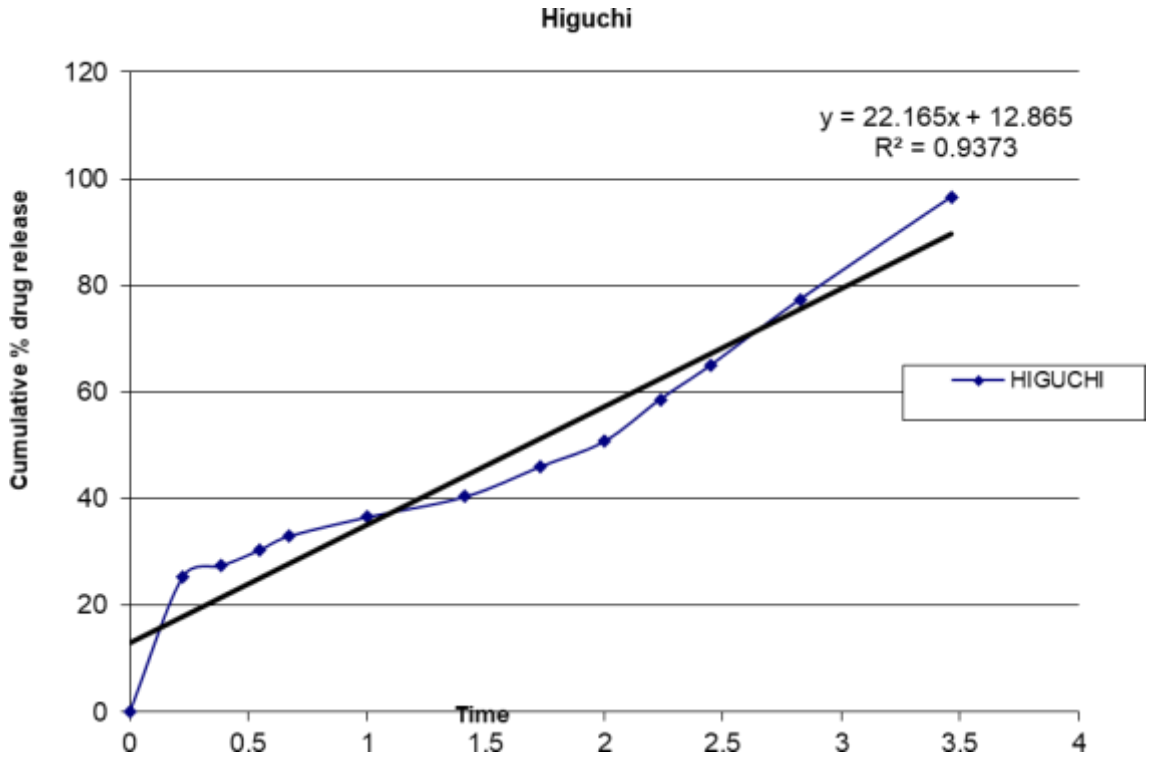
Figure 6: Standardisation of povidone iodine



IN-VITRO DRUG RELEASE KINETICS

In vitro drug release kinetic study was performed for the formulated dusting powder and the results are discussed in the table and graph was plotted. Formulated dusting powder has 96.57% of cumulative drug release at 12th hour. The Figure 7 shows that *invitro* drug release shows Higuchi model of release as it has regression value of 0.9373.

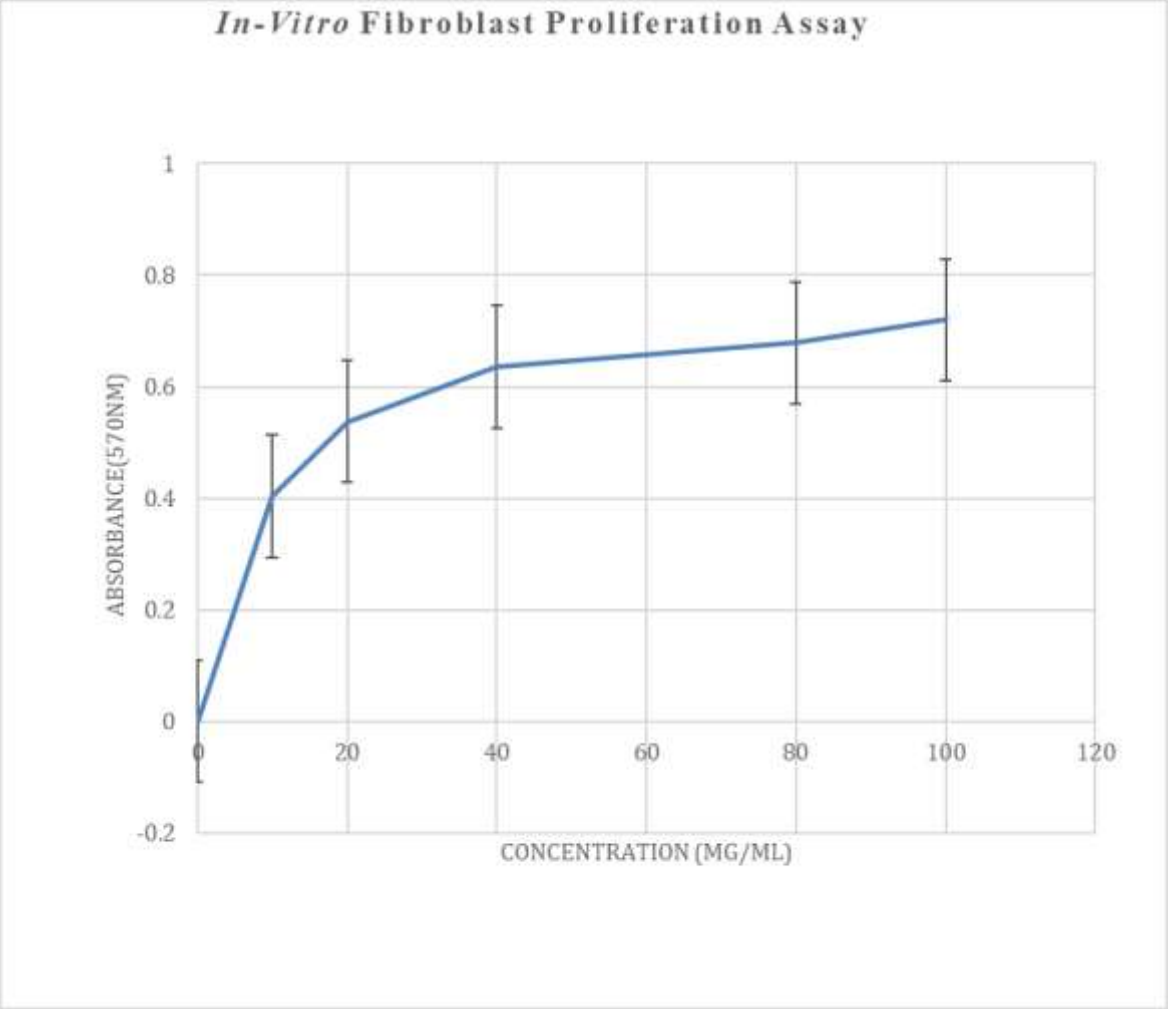
Figure 7: In – Vitro drug release kinetics of the formulation



3.7. IN-VITRO cell line assay

Among the various tested concentrations (10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml, 100µg/ml) at the dose of 100µg/ml the treated group produced significant proliferation when compared to that of the control (Figure 8). The result revealed that increase in concentration of the dusting powder causes increase in proliferation. Hence treated group showed concentration dependent proliferation in the fibroblast proliferation assay compared to that of the control group. Therefore 100µg/ml was found to be effective dose (ED).

FIGURE 8: *In Vitro* Fibroblast Proliferation Assay



3.8. *IN-VIVO* STUDY

3.8.1. MEASUREMENT OF FIN

Growth measurements and histological examination was done for the four experimental groups and one untreated group. Growth of the fin was measured using image J **1.52a software** the measurements were illustrated in the Figure 9, Figure 10.

Figure 9: Group I(control); **Group II** (collagen+ base); **Group III** (povidone iodine +base); **Group IV** (collagen + povidone iodine + base); GroupV (base).

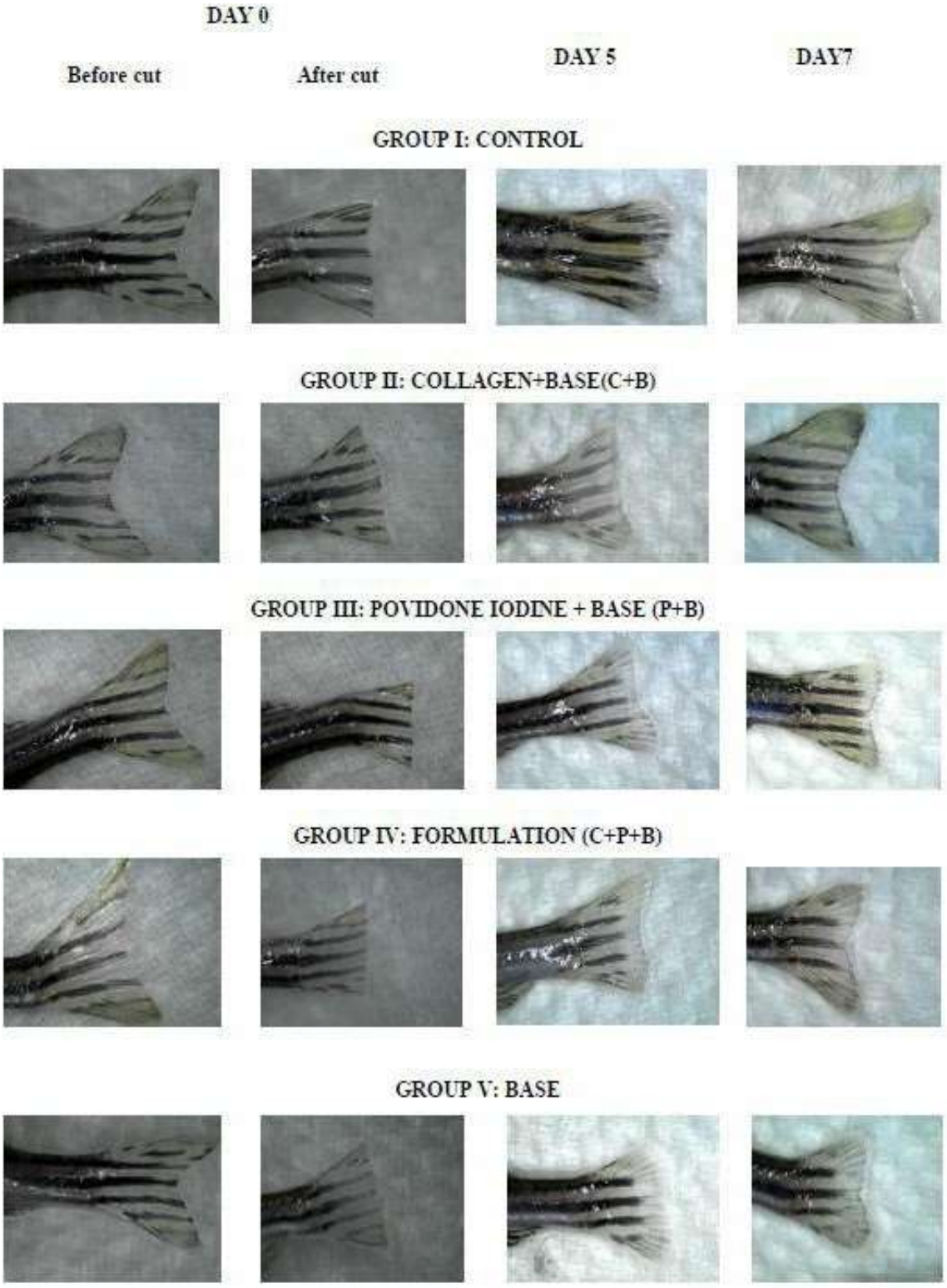
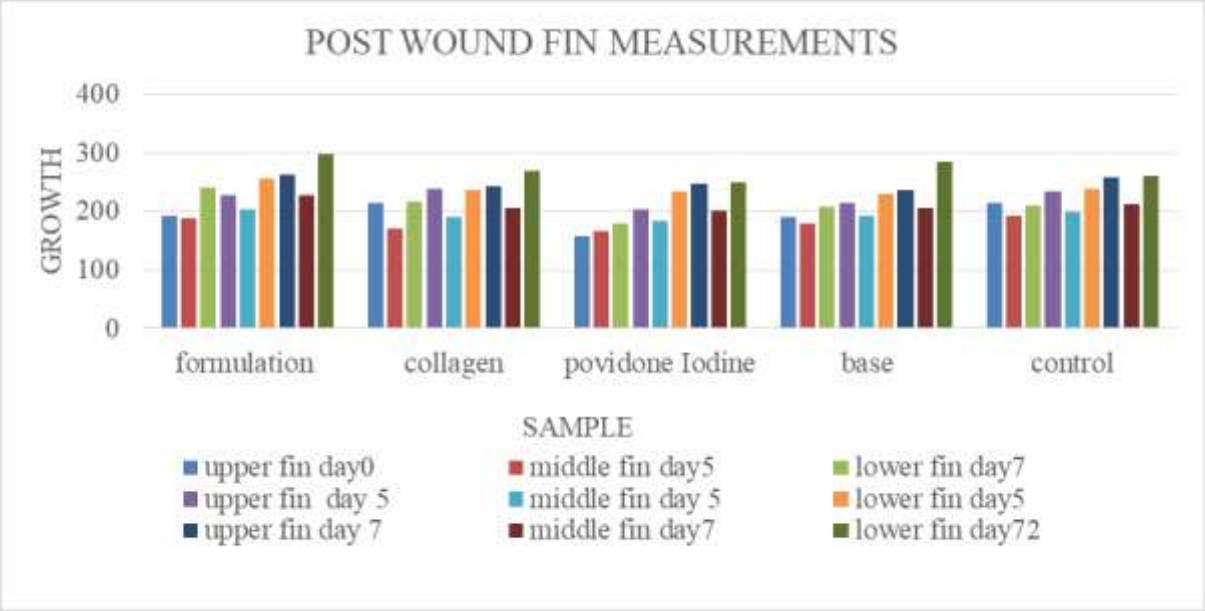


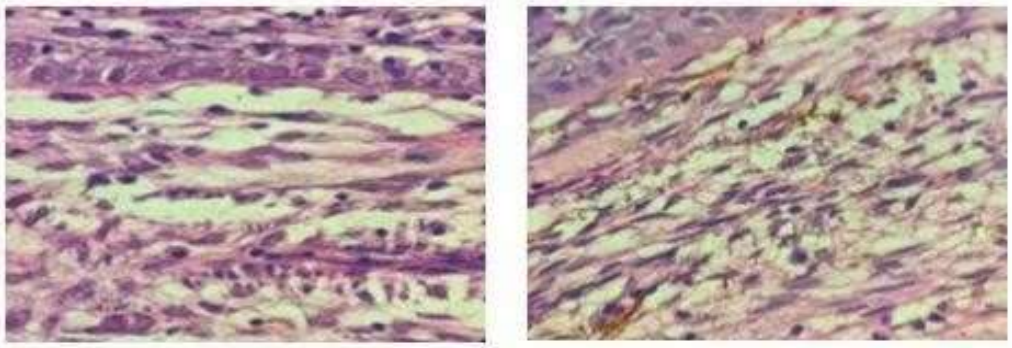
Figure 10: *In - vitro* fin measurement



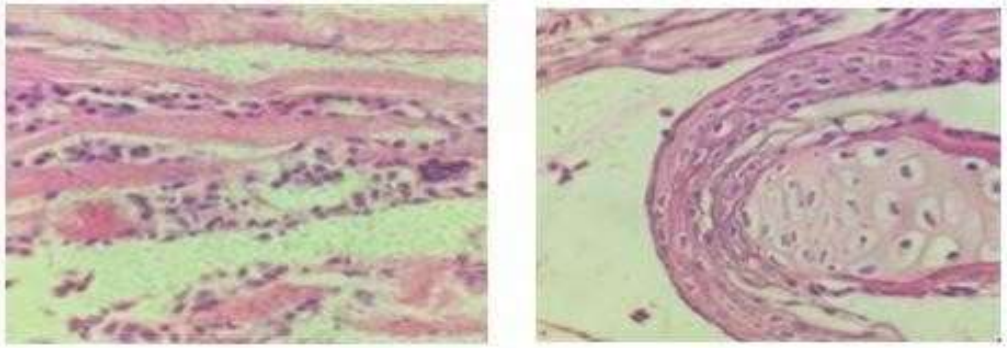
3.8.2. HISTOPATHOLOGICAL EXAMINATION:

Histopathological examinations of wound healing of treated and untreated group were illustrated in figure 11.

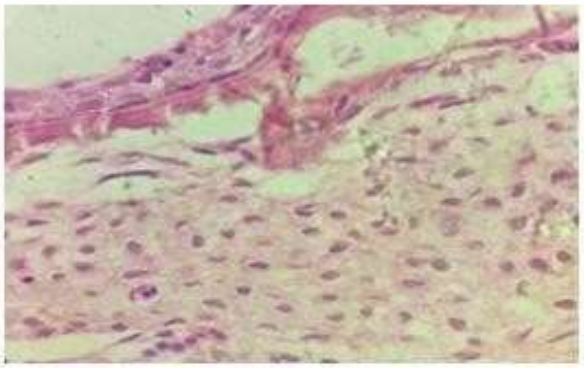
Figure 11: Histopathological perspective of wound healing in zebra fish



(a) (b)



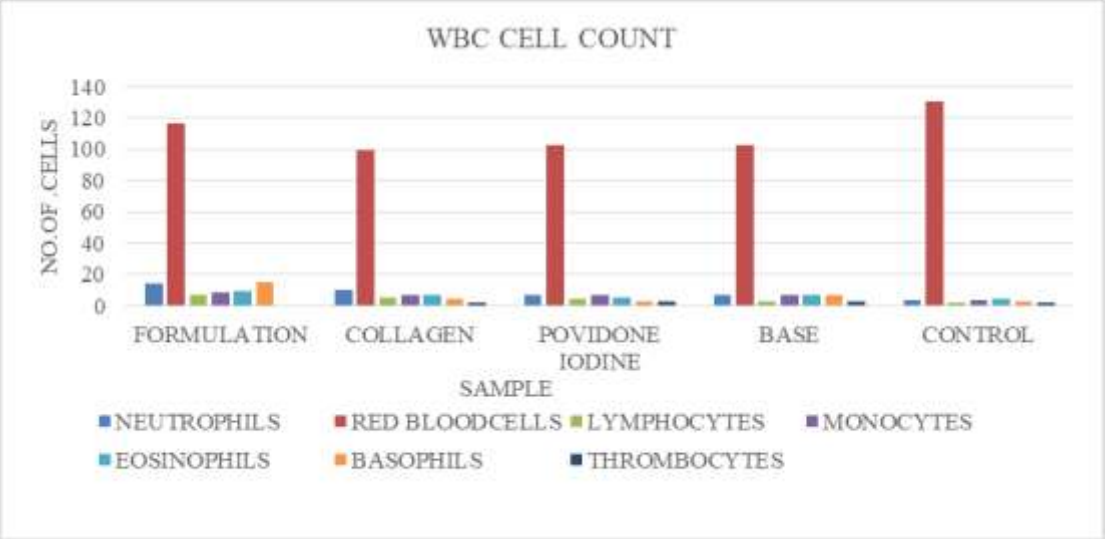
(c) (d)



(e)

A - control; **B** – collagen + base; **C -** povidone iodine + base; **D -** collagen + povidone iodine + base; **E –** base

Figure 12: In- vitro fin measurement



The results showed good re epithelialization of cells (Figure11). The formulated dusting powder treated group showed increased neutrophil count, where as other groups showed decreased number of neutrophil counts (Figure 12). The formulation containing collagen, povidone iodine and base (dusting powder) showed enhanced wound healing activity than the other groups. Povidone iodine loaded collagen-based dusting powder exhibit wound healing activity.

4. DISCUSSION

PVP loaded collagen-based dusting powder was formulated by trituration method. The angle of repose of dusting powder was determined using double open ended hallow cylinder and flow rate was found to be excellent. Water holding capacity carried out at 15minutes, 30minutes, 45minutes,1 hour. Formulated dusting powder has maximum water holding capacity at 30 minutes. FTIR spectra was obtained for collagen, povidone iodine, talc and chitosan and the spectra show there is no interaction between ingredients present in dusting powder. Formulation was subjected to temperature ranges from 20°C to 300°C and differential scanning calorimetry shows denaturation temperature at 103.6°C. Standardization of PVP of various concentration (0.01mg/ml,0.02mg/ml, 0.03mg/ml,0.04mg/ml,0.05mg/ml). Absorbance was measured in UV spectrophotometer at 240nm.Linearity was observed. Assay of povidone iodine in dusting powder was found to be 2.334. In *in vitro* release kinetic showed maximum cumulative percentage yield as 96.574% at 12^{th} hour and it follows Higuchi model of release kinetics. The cell proliferation assay was performed using the HDF (HUMAN DERMAL FIBROBLAST)

cell line. The cell proliferation assay was carried out with different concentrations of formulated dusting powder. The results revealed that formulation dusting powder showed increased cell proliferation at a maximum concentration of 100µg/ml. In *in vivo* zebra fish model, the wound was induced using fin amputation method and growth was observed on day 0, day 5, day 7 of various testing groups. Histopathological examination showed enhanced WBC counts. Formulated dusting powder showed potential wound healing in zebra fish.

5. CONCLUSION

The formulated dusting powder was characterized and evaluated for its efficacy towards the wound healing using *invitro* and *in vivo* methods. The result revealed that the prepared formulation has a potential wound healing effect, however further studies have to be carried out to confirm its mechanism of action to prove its wound healing efficacy.

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