Aminated magnesium hydroxide anchored curcumin- PVA nanofibers for cancer therapy

Vijayakumar Elumalai, Ravikant Gupta, Ranjithabala Venkatarajan, Monisha Thirumoorthy, J Prasanna, Dharmalingam Sangeetha*

Department of Mechanical Engineering, Anna University, Chennai 600 025, India
Corresponding author:sangeetha@annauniv.edu
Phone: +91- 44- 2235 7763

Abstract: Drug release profile of curcumin loaded aminated magnesium hydroxide (Mg $(OH)_2$) incorporated within PVA nanofibers for application in cancer therapy was reported in this study. The amination of Mg $(OH)_2$ was carried out using trimethoxysilane and was confirmed using FTIR. The drug (curcumin) was anchored onto the aminated $Mg(OH)_2$ ($Mg(OH)_2.NH_2$) by a simple chemical process. The above prepared curcumin- $Mg(OH)_2.NH_2$ complex was then incorporated (at 10 wt%) into PVA solution which was then electrospun to to form drug loaded nanofibers. The surface morphology of the fabricated nanofibers was studied under scanning electron microscope. The drug release study, performed using acetate buffer with different pH (3 and 5) and with phosphate buffer (pH 7.4), showed that the drug release rate was sustained and increased with decreasing pH as against the uniform release rate observed with PVA nanofibers loaded with curcumin without $Mg(OH)_2.NH_2$. The *in vitro* biocompatibility tests performed using HeLa and L929 cell lines showed good biocompatibility and anticancer effect on cancer lines. The study suggested that the novel preparation of curcumin - $Mg(OH)_2.NH_2$ loaded nanofibers have good potential for use in cancer treatment in areas with low pH.

Keywords: Aminated magnesium hydroxide, Curcumin, Electrospinning, PVA nanofibers.

I. INTRODUCTION

Cancer treatment has been carried out through various complicated therapies which always pose a problem of side effect to human body irrespective of the type and effect of the drug. Drug delivery proves to be one of the most eminent methods to overcome these effects[1]. Drug delivery has always been a topic of interest of the researchers[2-5], but the progress in targeted drug delivery is quite slow.

There are many forms employed for the drug delivery like beads, membrane and nano fibers among which the nano fibers have advantages of higher surface area. Electrospinning is one of the most efficient ways for producing polymer nanofibers of uniform diameter [6-7]. With improved and optimum parameters of the electro-spinner, fibers with very least diameter in the range of nano meter can be achieved without beads [8-10]. Thus this method has been proven to be the most preferred one for drug delivery application. Drug delivered from biodegradable polymer nano fibers has achieved high entrapment and sustained release rate [11-14]. Further, their improved therapeutic effect and high level of biocompatibility renders them as efficient carriers of antitumor drugs [15-16].

Antitumor drugs have become one of the most fast growing areas of interest of the researchers. Among them, curcumin takes the lead because of its cost effectiveness, efficiency and high levels of biocompatibility and Pharmacological safety [17-18]. The biodegradable polymer that has been used in this study is polyvinyl alcohol (PVA). It's excellent swelling property and biocompatibility combined with cost effectiveness has made it a fascinating material to be electrospun for the drug delivery application [19-20]. The PVA nano fibers act as efficient carriers for drug delivery. Since, curcumin is a hydrophobic drug, it is anchored with aminated magnesium hydroxide to enhance its release and achieve targeted delivery towards the acidic tumour [21]. In aminated magnesium hydroxide, hydrogen bonding takes place between the amine group and carbonyl group of curcumin which results in better anchoring of the drug with magnesium hydroxide.

In this present study, we fabricated and tested a novel controlled drug release system based on PVA nanofibers and curcumin anchored aminated magnesium hydroxide (cum-ami$Mg(OH)_2$) by electrospinning technique. The drug anchored magnesium hydroxide encapsulated within PVA nanofibers prolongs the release of the drug.

II. MATERIALS AND METHODS

PVA with molecular weight of 1,10,000 was purchased from B-pura, magnesium hydroxide ($Mg(OH)_2$) from Avra, India, 3-Aminopropyltriethoxysilane (Aldrich) and solvents chloroform, N,N-Dimethyl formamide (DMF) and ethanol were purchased from SRL and double distilled water was used throughout the experiment.

Preparation of functionalised magnesium Hydroxide

Magnesium hydroxide was functionalized using the protocol described by Gou etal [21]**.** 1gram of $Mg(OH)_2$ was dispersed in anhydrous N,N-dimethylformamide (DMF, 15mL) in a round bottom flask, 3-aminoproplytriethoxysilane (1mL) was added in drops to the solution for surface functionalization. The reaction mixture was stirred at 110^o C for 2 hours on an oil bath. The solution was then filtered using a whatman filter paper. The amine functionalized $Mg(OH)_2$ ($Mg(OH)_2$-NH_2) was recovered and dried for a day, at room temperature.

Pre-treatment of curcumin

Curcumin is a hydrophobic polyphenol derived from turmeric. Chemically, it is a bis-α,β-unsaturated β-diketone

(commonly called diferuloylmethane) that exhibits keto-enol tautomerism. Commercial curcumin is a mixture of curcuminoids, containing approximately 77% diferuloylmethane, 18% demethoxycurcumin, was used and 5% bisdemethoxycurcumin [17]. Pure turmeric which is a natural source of curcumin, was used as the source of curcumin in the present study. The turmeric was powered and was sterilized under ultraviolet medium for one day, before use [22-24].

Anchoring curcumin with $Mg(OH)_2$

$Mg(OH)_2$-NH_2 500mg was dispersed in ethanol. To this curcumin in ethanol solution (5ml, 100 mg/mL) was added [21]. The solution was stirred at room temperature for 12 hours. Then, the red-oranged product was filtered in whatman filter paper and washed with ethanol to remove the excess curcumin.

Fabrication of the drug loaded PVAnanofiber mat

1g of PVA was dissolved in 10 mL of cold distilled water, to obtain a homogeneous solution. 50 mg of curcumin was added to the polymer solution and stirred for 1 hour to obtain a homogenous solution. The homogenous solution was taken in 2.5 ml syringe with nozzle diameter of 0.55 mm, the solution was electrospun under 25 kV at a feed rate of 0.5 ml/h in an electrospinner machine to obtain the nanofiber mat Similarly, electrospun cum-ami$Mg(OH)_2$ loaded PVA mat was also obtained with loading of 100 mg of cum-ami $Mg(OH)_2$. The electro-spinning was carried out in electro-spinner (Pico-Chennai, India).

Fourier transforms infrared (FTIR) Spectroscopy

The FTIR analysis was conducted with Alpha Bruker in the spectral range from 4000 to 500 cm^{-1} to confirm the functional groups and to determine interaction between curcumin and aminated Mg $(OH)_2$.

Scanning electron microscopy (SEM)

Scanning Electron Microscope (Hitachi S – 3400 N) was used to analyse the surface morphology of the prepared nano fiber mats. The samples were cut into sufficient size and sputter coated with gold to make the samples electro conductive. The samples were then analysed under vacuum condition at an accelerating voltage of 10 kV.

Drug release kinetics

The drug loaded nanofiber was immersed in 5 mL buffer solutions of varying pH (pH 3, 5, 7). The amount of drug released was then determined spectrophotometrically using UV visible spectrophotometer (T90+ Uv/vis spectrometer PG instruments) at various time intervals. To determine the amount of drug released, a standard curve of curcumin in distilled water in different concentrations was plotted.

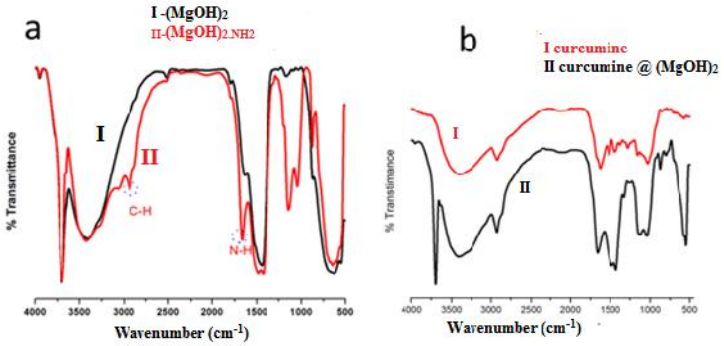
Biocompatibility studies

In vitro MTT ((3-[4,5-dimethylthialzol-2-yl]-2,5-diphenyltetrazolium bromide) salt assay test was used to analyse the biocompatibility of the prepared samples [26]. Cytotoxicity studies using the drug loaded fiber and the drug were analyzed in 96-well plate by MTT assay. L929, HeLa cell lines were cultured using 10 % fetal bovine serum then seeded into the tissue culture flasks. The L929, HeLa cell lines prepared at a density of 5000-7000 cells per well at 35º C. MTT assay solution was then added to each well and the plates incubated at 37º C for 3 h. Finally, the absorbance was measured using an ELISA reader for 24 hrs and 36 hrs.

III. Results and Discussions

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of $Mg(OH)_2$ and amine functionalized $Mg(OH)_2$ is shown in fig 3.1a sharp and intense vibration band at 3697 cm^{-1}, can be attributed to the O-H stretching in the crystal structure of $Mg(OH)_2$ and a strong band at around 500 cm^{-1} can be related to the Mg-O. The incorporation of amine group in $Mg(OH)_2$ can be qualitatively confirmed by the two new bands assigned to CH_2 and N-H that appeared at 2921 cm^{-1} and at 1670 cm^{-1} respectively. This result confirmed that the surface was successfully functionalized with amine group [21] . Fig 3.1b shows the curcumin and aminated magnesium hydroxide anchored with curcumin. In curcumin spectrum, the presence of peaks at 1625 cm^{-1} showed the presence of carbonyl groups (C=O) of curcumin and the vibrational bands at around 1034 cm^{-1} can be due to C-O stretching. For the drug loaded amine functionalised $Mg(OH)_2$ the carbonyl group disappears and N-H stretching intensity decreases this confirms the hydrogen bonding between the C=O of curcumin and amine group of $Mg(OH)_2$.



FTIR spectrum of 3.1a $Mg(OH)_2$, $Mg(OH)_2.NH_2$ and 3.1b curcumin ,drug loaded $Mg(OH)_2.NH_2$

Scanning Electron Microscopy (SEM)

Figure 3.2 shows the SEM images of the electro spun nano fiber mats. The image corresponding to PVA with curcumin (Fig 3.2 a) shows evidence of encapsulation of the drug within the nanofibers. The fibers were of uniform diameter and no beads were observed. The average size of the fibers was in the range of 300 and 650 nm.Whereas, in the SEM image corresponding to cum-ami $Mg(OH)_2$ loaded PVA nanofiber mat, the morphology of the fibers appeared to have changed. This difference was attributed to the action of $Mg(OH)_2$

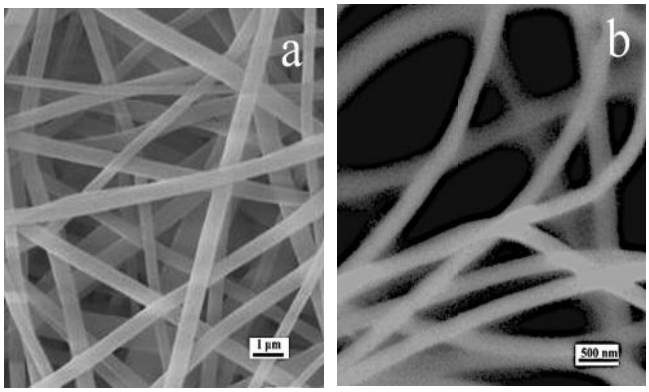


Fig.3.2 SEM images of PVA nanofiber mat with curcumin (a) and cum-ami $Mg(OH)_2$ loaded PVA nanofiber mat (b)

DRUG RELEASE KINETICS

The *in vitro* drug release profile of curcumin and cum-ami$Mg(OH)_2$ loaded PVA nanofiber mats in PBS at various pH of 3, 5 and 7.4 (simulating normal body fluid) is shown in Fig.3.3 and Fig.3.4. Two stages were observed at all pH. In the first stage, an initial burst release was observed which lasted for the first 30 minutes. This was due to the immediate release of the drug present on the surface of the nanofiber when exposed to an environment of a different pH from its own. It was then followed by a phase of sustained release which was attributed to the release of the entrapped drug inside the nanofiber (second stage). This phase lasted approximately for 90 minutes. Finally, at the end of 180 minute, the complete dissolution of the polymer resulted in the complete release of the drug. MingyiGuo et al[21] reported without the polymer matrix aminated $Mg(OH)_2$ shows the life span of about 60 min in the present work due the polymer role the life span increases to 180 min.

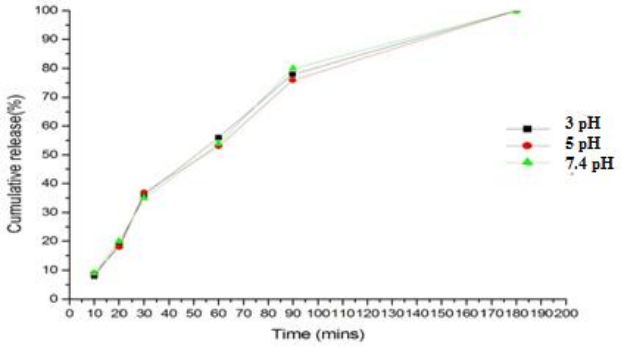


Fig.3.3 Curcumin profile

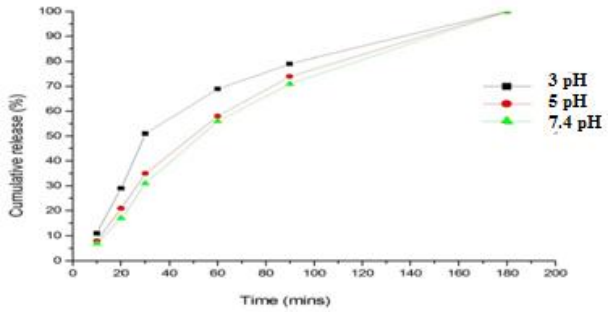


Fig. 3.4 Curcumin anchored $Mg(OH)_2.NH_2$ drug realise profile

The drug release profile of cum-ami$Mg(OH)_2$ has shown significant difference from that of curcumin profile at different pH. In contrast to the drug release rate of curcumin, the drug release rate of cum-ami$Mg(OH)_2$ showed an interesting and varied release profile at various pH. At pH 3, the burst release was the maximum as compared with the other two pH and was observed within 30 minutes accounting for 50% of the drug release. However in pH 7.4, a decreased rate of release was observed but was moderate at pH 5. In all the buffer solutions, a sustained release followed until 100 minutes and later release was attributed to the dissolution of the polymer. It should be noted that the amount of drug release at all times was higher in case of pH 3 when compared with the other two pH. This observed higher release in acidic pH and retarded release in basic pH was attributed to the basicity of $Mg(OH)_2$. The result of the study shows that this property could be exploited to fabricate pH responsive drug release systems. Further, the time of entire drug release was prolonged to 3 hours from one hour in the work of MingyiGuo et al[21].

MTT ASSAY

The results of MTT assay showed that the amount of viable cells in the normal cell (L929) lines was higher when compared with that of the cancer cells, this indicated that the drug was toxic to the cancer cells than the normal cells . The cell viability was observed to be more than 90% and 85% in the case of normal cells for the drug loaded fiber and simply the drug respectively. Thus the entire biomaterial (cum-ami$Mg(OH)_2$PVA nanofiber) used was proved to be biocompatible. It was found that the cell viability decreased to less than 85% for the cancer cells. Further, an investigation of the morphology of the cells was done through optical microscope in the fluorescence mode which proved that the cell viability of the cancer cells reduced with an associated change in morphology.

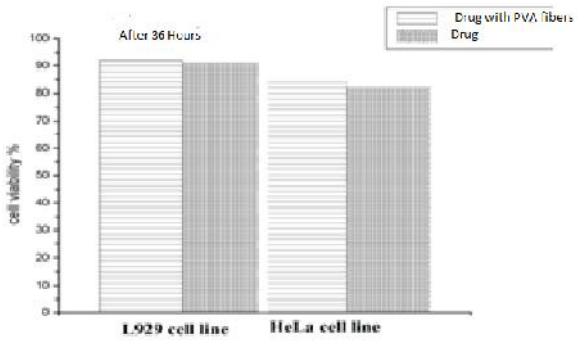


Fig.3.5 MTT assay results after 36h

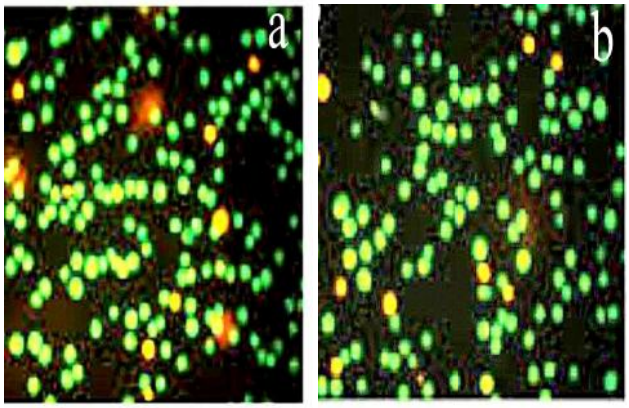


Fig.3.6 Microscopic images of L929 cell lines used before (a) and after test (b)

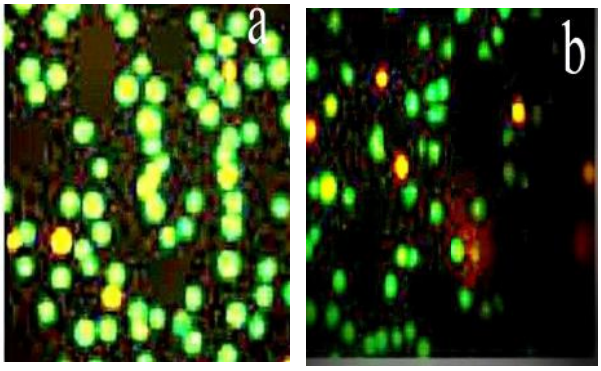


Fig.3.7 Microscopic images of HeLa cell lines used before (a) and after test (b)

IV. CONCLUSION

A biocompatible polyvinylalcohol (PVA) polymer nanofiber mat was prepared with curcumin and curcumin anchored aminated magnesium hydroxide, after studying the parametric influence on the fiber formation. They were characterized using FTIR and SEM which confirmed the presence of the drug within the PVA nanofiber matrix. On observation of the drug release profile, it was inferred that the drug anchored with aminated magnesium hydroxide was pH responsive. Further, the time required to effect entire drug release was prolonged to 3 hours from one hour as reported by Mingyi Guo et al [21]. The MTT assay test confirmed that the polymer nanofiber mat and the drug were not cytotoxic for normal cell line (L929), but toxic to cancer cell lines (HeLa). The thus fabricated curcumin -$Mg(OH)_2.NH_2$ complex loaded PVA nanofibers show promise in the treatment of cancer

ACKNOWLEDGMENT

The authors would like to thank Council of Scientific and industrial research (CSIR), New Delhi, India for funding the study (Vide letter No.01(2452)/11/EMR-11, letter dated 16.05.2011).

REFERENCES

[1] S.Prakas, M.Malhotra, W.Shao, C.T.Duchesneau, and S.Abbasi, "Polymeric nanohybrids and functionalized carbon nanotubes as drug delivery carriers for cancer therapy,"Advanced drug delivery reviews,vol 63, pp. 1340-1351,Nov2011.

[2] M. Sui, W. Liu, and Y. Shen, "Nuclear drug delivery for cancer chemotherapy,"Journal of Controlled Release, vol 155, pp. 227–236, Oct 2011

[3] S.Biswas, P.Vladimir, and Torchilin "Nanopreparations for organelle-specific delivery in cancer," Advanced Drug Delivery Reviews,vol 66, pp. 26–41,Jan 2014

[4] K.Cho ,X.Wang , S.Nie , D.M Shin "Therapeutic Nanoparticles for Drug Delivery in Cancer". Clinical Cancer Research. vol. 14, pp. 1310-1316, May 2008.

[5] V. Torchilin, "Tumor delivery of macromolecular drugs based on the EPR effect," Advance .Drug Delivery. Review.vol 63 pp. 131–135, Jul 2011

[6], B. Cramariuc, R. Cramariuc, R. Scarlet,and G. Lupu,Oana, "Fiber diameter in electrospinning process" Journal of Electrostatics vol71 pp. 189-198, Jun 2013

[7]Nandana Bhardwaj, and S. C. Kundu "Electrospinning: a fascinating fiber fabrication technique," Biotechnology Advances vol28 , pp. 325-347 Jan 2010

[8] P. Heikkil, and A.Harlin,"Parameter study of electrospinning of polyamide-6," European Polymer Journal vol 44 pp. 3067–3079 Jun 2008

[9] C.J. Thompson, G.G. Chase, A.L. Yarin, and D.H. Reneker, "Effect of parameters on nanofiber diameter determined from electrospinning model,"Polymer vol48, pp. 6913-6922, Mar 2007

[10] J.M. Deitzel, J. Kleinmeyer,D. Harris, and N.C. Beck Tan, "The effect of processing variables on the morphology of electrospunnanofibers and textiles," Polymer vol. 42, pp. 261–272, Jan 2001

[11] T. J. Sill , Horse and A. V.Recom. "Electrospinning: Application in drug Delivery and tissue engineering.Biomaterials, vol. 29, pp. 1989 – 2006, Jun 2008

[12] A. J. Meinel, O.Germershaus, T. Luhmann, P. Markle, Lorenz, and Meinel. "Electrospun matrices for localized drug delivery: current technology and selected biomedical applications" Biopharmaceutics vol. 81, pp. 1 – 13, May 2012.

[13] A. Frenot, and I. S. Chronakis, "Polymer nanofibers assembled by electrospinning."Current Opinion in Colloid and Interface Science vol 8 pp. 64–75, Aug 2003.

[14]M.Hamori, ShioriYoshimatsu,YHukuchi, Yuki Shimizua, KeizoFukushimab, Nobuyuki Sugiokab, Asako Nishimura, and Nobuhito Shibata, "Preparation and pharmaceutical evaluation of nano-fiber matrix supported drug delivery system using the solvent-based electrospinning method,"International Journal of Pharmaceutics
Vol 464, pp. 243–251, Apr 2014

[15] D Sangeetha,and S. S.Sundar, "Fabrication and evaluation of electrospun collagen/ poly(N-isopropylacryamide)/ chitosan mat as blood contacting biomaterials for drug delivery," Mater Med vol. 23, pp. 1421–1430. Jun 2012.

[16] D Sangeetha, and S. S. Sundar, "Investigation on sulphonated PEEK beads for drug delivery, bioactivity and tissue engineering applications". J Mater Sci., vol. 47pp. 2736–2742, Dec 2012

[17] P. Anand, C. Sundaram, S. Jhurani, A. B. Kunnumakkara, and B. B. Aggarwal, "Curcumin and cancer: An ''old-age" disease with an ''age-old" solution" Cancer Letters vol. 267,pp.133–164, Mar 2008

[18]B. Chen, Y. Zhang, Y. Wang,J.Rao,X.Jiang,andZ. Xu, "Curcumin inhibits proliferation of breast cancer cells through Nrf2-mediated down-regulation of Fen1 expression,"The Journal of Steroid Biochemistry and Molecular Biologyvol. 143, Pages 11–18, Sep 2014.

[19] Ji. S. Im , J. Yun , Y. M. Lim , H. Kim, and Y. Seak "Fluorination of electrospun hydrogel fibres for a control release drugdelivery system," ActaBiomaterialia vol. 6 pp. 102 – 109, Jan 2012

[20] X.Jin , and Y. L. Hsieh "PH-responsive swelling behavior of poly(vinyl alcohol)/poly(acrylic acid) bi-component fibrous hydrogel membranes,".vol. 46:5149–5160 May 2005

[21] M. Guo, F. Muhammad, A. Wang, W. Qi, N. Wang, Y. Guo, Y. Wei and G. Zhu "Magnesium hydroxide nanoplates : a pH responsive platform for hydrophobic anticancer drug delivery "J. Mater. Chem. B, vol.1, pp. 5273-5278, Apr. 2013
.
[22],C.C. Lin, H.Y. Lin, M.H. Chi, C. M. Shen,H.W. Chen, W.J. Yang, and M.H. Lee , "Preparation of curcumin micro-emulsions with food-grade soybean oil/lecithin and their cytotoxicity on the HepG2 cell line," Food Chemistry,Vol.154, , pp. 282–290, July 2014.

[23]GangjianJi, J. Yang, andJ. Chen, "Preparation of novel curcumin-loaded multifunctional nanodroplets for combining ultrasonic development and targeted chemotherapy,"International Journal of Pharmaceutics, vol. 466, , pp. 314–320, May 2014.

[24]W. H. Khan, V. K. Rathod "Process intensification approach for preparation of curcumin nanoparticle via solvent-nonsolventnanoprecipitation using spinning disc reactor," Chemical Engineering and Processing: Process Intensification, vol. 80, pp. 1–10, Jun 2014.

[25] Kokubo, T. & Takadama, How useful is SBF in predicting in vivo bone bioactivity. Biomaterials, vol 27, pp,2907-2915 May 2006.