In vivo anti-obesity efficacy of curcumin loaded nanofibers transdermal patches in high-fat diet induced obese rats

Amir reza Ariamoghaddam a, Bahman Ebrahimi-Hosseinzadeh b,⁎
Ashrafalsadat Hatamian-Zarmi a,⁎⁎, Razi Sahraeian b

a Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, P.O. Box 14395-1561, Tehran, Iran
b Department of Composite Engineering and Processing, Iran Polymer Institute, Tehran, Iran

A R T I C L E   I N F O

Keywords:
Transdermal delivery
Nano
Fiber
Electrospinning
Adipose tissue
MRI imaging
Obesity

A B S T R A C T

Obesity as a dominant problem in developed countries which is known to be basic step of so many diseases is subjected to find a solution for in this work. Curcumin containing polyvinyl alcohol-gelatin nanofibers which ranging from 200 to 250 nm in diameter as a transdermal drug delivery system for declining volume of subcutaneous adipose tissue is investigated here. Morphology and synthesis method of nanofibers is designed and optimized by statistical software and a totally uniform and reproducible method of synthesis is used for preparation of a transdermal patch. Effectiveness of delivery system in transport of drug through skin is confirmed by side by side arrangement transdermal diffusion cells. This transdermal patch used for animal test showed 4 to 7% decrease in total amount of adipose tissue estimated by whole body magnetic resonance imaging technique.

1. Introduction

Transdermal methods as a new delivery method for drugs started to attract attentions since 1980s [1]. This method of delivery has advantages over oral or injection method of delivery. It is less aggressive rather than injection and its advantage over oral delivery is that drug is not force to pass through harsh environment of gastrointestinal system. In addition, its spread over blood circulatory system is less effective by liver and kidney function [1]. However, since that time, there are few examples of commercial transdermal drug delivery products. The major limitation of this delivery method is impermeability of most outer layer of skin called stratum corneum. For this reason, drug molecules used to be delivered should not be so large (400–500 Da) and a balanced lipophilicity (log octanol-water partition coefficient around 2 to 3 ideally) [2] is required for it to get into the blood stream. In addition, dose of drug required to be effective should not be so high [2].

Some drugs have been FDA approved to be used in transdermal delivery systems. Among them scopolamine (1979), glyceryl trinitrate (1981), clonidine (1984), estradiol (1986), fentanyl (1990), nicotine (1991), testosterone (1993), estradiol & norethisterone acetate (1998), norelgestromin & ethinyl estradiol (2001), estradiol & levonorgestrel (2003), oxybutynin (2003), selegiline (2006), methylphenidate (2006), rotigotine (2007), rivastigmine (2007) and granisetron (2008) are commercialized [2]. For heavier drugs and molecules which don’t pass through skin easily, introduction of microneedles into transdermal patches has shown to be effective for a wide range of drugs and even vaccines since it bypass barrier layer mechanically [3, 4]. However, because of its moderate aggressiveness, in cases where drug delivery is possible without use of these semi aggressive methods, it seems to be preferable.

For treatment of obesity a wide range of treatments are advised. Some of them refer to change of lifestyle of patients which is the main cause of appearance of disease [5]. There is other type of methods including surgery which is very aggressive and in most cases reversible. Orally use of drugs which affects digestion of high calorie content of intake food has been approved by FDA. For example, Orlistat inhibits lipases in gastrointestinal system and prevents digestion of fats. However, it has its own side effects too. In searching for more effective method with less side effects, transdermal delivery treatment of obese mice have been studied using polymeric microneedle patches [6]. It is shown to be effective in decreasing volume of adipose tissue in diet induced obese mice. In addition, there are other effective drugs which use of them seems to be less aggressive. For example there are several phytochemical compounds which has been proved to effect as an anti-obesity agent on this type of tissue but for them to be effective high amount of them should be used orally due to kidney clearance function [7]. Resveratrol [8, 9], EGCG [10], genistein [11], and curcumin [12–16] are example of these phytochemicals which their metabolic function have been studied extensively.

As a drug which can target adipose tissue, curcumin, a yellow...
A. R. Ariamoghaddam et al.


**Table 1**

Design parameter of screening model.

<table>
<thead>
<tr>
<th>Run</th>
<th>Flow rate (ml/h)</th>
<th>Voltage (kV)</th>
<th>PVA %</th>
<th>Gel %</th>
<th>AA %</th>
<th>Distance (cm)</th>
<th>Drum speed (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.20</td>
<td>15,000</td>
<td>4</td>
<td>15</td>
<td>5</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>1.20</td>
<td>25,000</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>0.30</td>
<td>15,000</td>
<td>1</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>0.30</td>
<td>15,000</td>
<td>4</td>
<td>8</td>
<td>15</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>1.20</td>
<td>25,000</td>
<td>1</td>
<td>8</td>
<td>5</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>0.30</td>
<td>15,000</td>
<td>1</td>
<td>15</td>
<td>5</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>0.30</td>
<td>25,000</td>
<td>4</td>
<td>15</td>
<td>5</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>1.20</td>
<td>15,000</td>
<td>1</td>
<td>8</td>
<td>15</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>0.30</td>
<td>25,000</td>
<td>4</td>
<td>8</td>
<td>15</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>1.20</td>
<td>15,000</td>
<td>4</td>
<td>15</td>
<td>5</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>1.20</td>
<td>25,000</td>
<td>1</td>
<td>15</td>
<td>15</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>0.30</td>
<td>25,000</td>
<td>1</td>
<td>15</td>
<td>15</td>
<td>8</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2**

Statistical parameter of screening model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F value</th>
<th>p-Value Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent percent in gelatin solution</td>
<td>922.77</td>
<td>922.77</td>
<td>5.06</td>
<td>0.0678</td>
</tr>
<tr>
<td>Mix ratio (PVA/gelatin)</td>
<td>3238.48</td>
<td>3238.48</td>
<td>5.39</td>
<td>0.0074</td>
</tr>
<tr>
<td>Distance</td>
<td>1.40</td>
<td>1.40</td>
<td>18.93</td>
<td>0.9315</td>
</tr>
<tr>
<td>voltage</td>
<td>15.62</td>
<td>15.62</td>
<td>8.156E-003</td>
<td>0.7747</td>
</tr>
<tr>
<td>Drum speed</td>
<td>363.64</td>
<td>363.64</td>
<td>0.091</td>
<td>0.2047</td>
</tr>
<tr>
<td>Flow rate</td>
<td>648.90</td>
<td>648.90</td>
<td>2.13</td>
<td>0.1090</td>
</tr>
<tr>
<td>Model</td>
<td>5190.80</td>
<td>171.08</td>
<td>3.79</td>
<td>0.0480</td>
</tr>
</tbody>
</table>

For enhancement and control of drug delivery by transdermal method, various systems have been used. Use of thin polymer films as a layer on the skin was the first method for preparing delivery system [2]. However, for increasing the rate of delivery and decreasing time required to reach to a steady state, use of nanostructured materials is an option. Nanoparticles in different systems like liposomes [20], transfersomes [21], and many different nanostructured materials [1] have been used to increase the obtain better delivery results. Use of nanoparticles as a nanostructures system for transdermal drug delivery is rarely reported [22, 23]. Use of nanoparticles in transdermal delivery systems has advantages; low diameter of fibers increased the rate of drug diffusion from inside of fiber matrix to the surface of skin, obtaining very uniform distribution of drug and high repeatability of release profile gives a better control of synthesis in comparison to other nanostructured systems.

In this work, we used a blend of gelatin/albumin and PVA for entrapment of curcumin to obtain a transdermal delivery patch. PVA based nanofibers are previously investigated by some authors [24–26]. Blend of gelatin and curcumin is previously reported to be synthesized by electrospinning [27–29]. Here, we screened parameters in synthesis of fibers and selected the most effective parameters and then optimized fiber quality via controlling of synthesis parameters using sets of data. Using optimum point achieved by this method, drug delivery system to be tested ex vivo and in vivo for transdermal delivery and effectiveness in decreasing adipose tissue mass in rats. Weight profile of animals followed for each group in period of experiment. In addition to estimation of volume percent of adipose tissue by MRI imaging were used to confirm effectiveness of drug delivery system. Estimation of concentration of some obesity related metabolites in blood plasma used to confirm effectiveness of this delivery system to take drug molecule to target tissue.

**2. Materials and methods**

**2.1. Materials**

Bio-degradable PVA (poly vinyl alcohol) (MV = 89,000 to 98,000 and 99 + % hydrolyzed) and analytical grade gelatin was purchased from were purchased from Sigma-Aldrich (USA). All reagents and chemicals were purchased from Merck. Curcumin was purchase from Sigma-Aldrich.

**2.2. Screening electrospinning parameters for preparation of nanofiber**

The electrospinning of nanofibers was performed by an instrument made by Fanavaran Nano Meghias Company, IRIB. Condition for electrospinning was designed by design expert software based on Placket-Berman method. Twelve different conditions with various voltage, PVA concentration, gelatin concentration, formic acid concentration, distance between tip of needle and collector, injection speed and drum speed were used for preparation of nanofibers and SEM images of them were captured by Serontechnologies AIS2100 instrument in Amirkabir University, Tehran, Iran. Factors extracted from images used as response to analyze nanofiber synthesis method. These factors were diameter, diameter standard deviation, pore size, pore size standard deviation and porosity.
of fibers and encapsulation of drug inside it, its chemical properties were calculated by a Brucker IR spectrophotometer instrument.

2.6. Drug release in PBS buffer and NaCl solution

Patch containing drug was tested of drug profile release in PBS buffer. Determination of released drug concentration after each time interval was performed with UV–visible spectrometer and compared with calibration curve in multiple wavelengths. IR spectrum of drug containing, and drug free patch were gathered by Brucker IR spectrometer. Another release profile for diffusion of drug from drug containing nanofiber into a 0.1% (weight-weight) solution of NaCl is shown in Fig. 8.

2.7. In vitro transdermal drug delivery test

This test was performed by side by side diffusion cell system in a way that three sample of transdermal patch containing drug and one blank patch sample were setup using rat abdomen skin sample. Samples from each cell gathered in time interval and PBS buffer replaced. Concentration of drug evaluated by UV–vis spectrophotometer. Absorption results baseline corrected by results gathered from reference cell in totally similar condition unless absence of drug in transdermal patch and averaged for three repeats. Surface area of skin tested was 0.758 cm². Detailed description of this test is shown in Fig. 1.

2.8. Animal test

Three groups of rats, each with 6 members, aged 8 weeks were started to be prepared for animal test. The first groups with normal diet were used as negative control marked as “N”. The second two groups were receiving high calorie diet by adding fructose to their drinking water. After 4 months we had two obese groups of rats and a normal group. In this stage drug administrated on one group marked as “Dc”. The third groups of mice which were obese and were not receive drug were marked as “L”. Drug administration was as follows:

Each rat was shaved in abdomen region and a 4 cm² transdermal patch was attached to the area. During 6 weeks their weight were monitored and each week transdermal patch of each one was replaced. After this period of time body of each rat was analyzed using MRI (magnetic resonance imaging technique) based on reference method [31] to determine volume percent of adipose tissue in their body. MRI imaging performed by SIMENS 3 T MRI imaging instrument located at Iran National Brain Mapping Center of Iran, University of Tehran. MRI parameters were adjusted based on works of for obtaining more accurate results, separated tissue were used as standard and using ImageJ software, threshold require for separation of various tissue including muscle, adipose tissue and liver. All MRI obtained images of animal body were analyzed based on these threshold values and volume percent of adipose tissue determined for all images captured from body of rats of each group. Then, total volume fraction of fat for each animal calculated by a couple of codes summarizing all images of each sample. Their blood samples were gathered for concentration of triglycerides, cholesterol and leptin.

2.9. MRI imaging and data extraction

For determination of adipose tissue volume, from each group rats were selected and subjected to MRI whole body imaging based on reference protocol [31–33]. In addition, samples of muscle, liver, and adipose tissue of a rat just after postmortem were subjected to MRI imaging in test tube. Thresholds used to distinguish between these tissues for standard samples used for visual separation of adipose tissue in MRI images. All images processed with ImageJ software.

2.10. Statistical

Experimental design and statistical calculation of results performed by trial version of Design Expert software. Extraction of response parameters for nanofiber patches and statistical calculation of parameters including fiber diameter, fiber diameter standard deviation and porosity of fibers performed by Diameter J plug-in package of Image J software.

All release profile experiments performed three times and average values were reported. For animal test, each group of animal including control groups and drug receiving groups had at least 6 members. Reported data for weight profiles and chemical test in blood plasma were average values of all group members. Statistical analysis of results of animal test performed based on variance analysis method by SAS 9.1 software.

3. Results and discussion

The optimum point to be selected for drug loading was based on these points:

a. Decreasing time required for diffusion of drug from nanofiber matrix to the surface of skin.

b. Reaching maximum uniformity in fiber composition to obtain highest reproducibility in drug release.

As previously confirmed, encapsulation of curcumin into gelatin/
albumin nanoparticles increases its solubility dramatically [34]. Based on the fact that molecular mass and water-octanol partition coefficient of curcumin was suitable as a drug to be delivered transdermally [35], it seems that diffusion of curcumin from encapsulation vehicle to the skin surface is an important parameter in increasing transdermal diffusion flux of drug because this diffusion rate can be comparable to diffusion through *stratum corneum*. In this diffusion rate, process bottleneck is drug passage through outer skin layer.

To achieve these goals, three parameters were selected to be optimized; first, fiber diameter should be minimized to decrease diffusion time of curcumin to surrounding solution. Second, porosity of system should be maximized to enhance fiber wetting and decrease time of drug diffusion from fiber surface to the skin surface. Third, fiber diameter standard deviation to reach maximum uniformity.

Table 1 shows the parameters to be screened for nanofiber synthesis optimization based on previously described parameters. Three main components including gelatin solved in formic acid, PVA solved in distilled water and ethanol (as the drug solvent) were used to prepare electrospinning initial. Other parameters including, voltage, drum speed, distance between needle tip and drum, and flow rate were selected to be screened. Among all parameters as what can be observed, formic acid percent, PVA percent in addition to voltage are the most effective parameters in decreasing standard deviation of fiber diameter and patch porosity. Voltage is the only factor which affects porosity, while increasing mix ratio in favor of PVA rather than gelatin leads to more uniform fibers with fewer beads. Meanwhile, gelatin is chosen to be blended with PVA. PVA selected for two reasons; first, it is cheap and

<table>
<thead>
<tr>
<th>Run</th>
<th>Mix ratio</th>
<th>Formic acid (%)</th>
<th>Voltage (kV)</th>
<th>Diameter (nm)</th>
<th>Diameter standard deviation</th>
<th>Porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.526</td>
<td>90</td>
<td>22</td>
<td>294.8</td>
<td>79.5</td>
<td>0.4728</td>
</tr>
<tr>
<td>2</td>
<td>0.526</td>
<td>90</td>
<td>22</td>
<td>274</td>
<td>75</td>
<td>0.4731</td>
</tr>
<tr>
<td>3</td>
<td>0.526</td>
<td>95</td>
<td>17</td>
<td>295</td>
<td>81.8</td>
<td>0.484</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>85</td>
<td>22</td>
<td>226.1</td>
<td>53.9</td>
<td>0.474</td>
</tr>
<tr>
<td>5</td>
<td>0.526</td>
<td>85</td>
<td>27</td>
<td>222.1</td>
<td>58</td>
<td>0.471</td>
</tr>
<tr>
<td>6</td>
<td>0.526</td>
<td>80</td>
<td>22</td>
<td>299.9</td>
<td>77.9</td>
<td>0.4729</td>
</tr>
<tr>
<td>7</td>
<td>0.526</td>
<td>85</td>
<td>17</td>
<td>223.7</td>
<td>39.9</td>
<td>0.4681</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>90</td>
<td>17</td>
<td>222.5</td>
<td>53.6</td>
<td>0.4686</td>
</tr>
<tr>
<td>9</td>
<td>0.052</td>
<td>95</td>
<td>22</td>
<td>271.8</td>
<td>73.1</td>
<td>0.4686</td>
</tr>
<tr>
<td>10</td>
<td>0.052</td>
<td>95</td>
<td>22</td>
<td>268.4</td>
<td>71.6</td>
<td>0.4735</td>
</tr>
<tr>
<td>11</td>
<td>0.526</td>
<td>95</td>
<td>27</td>
<td>237.5</td>
<td>40.9</td>
<td>0.4793</td>
</tr>
<tr>
<td>12</td>
<td>0.052</td>
<td>90</td>
<td>27</td>
<td>245.3</td>
<td>65.9</td>
<td>0.4798</td>
</tr>
<tr>
<td>13</td>
<td>0.052</td>
<td>90</td>
<td>17</td>
<td>260.3</td>
<td>86.7</td>
<td>0.478</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>90</td>
<td>27</td>
<td>241.7</td>
<td>101.3</td>
<td>0.4811</td>
</tr>
<tr>
<td>15</td>
<td>0.052</td>
<td>85</td>
<td>22</td>
<td>235.8</td>
<td>50.1</td>
<td>0.4639</td>
</tr>
<tr>
<td>16</td>
<td>0.526</td>
<td>90</td>
<td>22</td>
<td>296</td>
<td>83</td>
<td>0.4732</td>
</tr>
<tr>
<td>17</td>
<td>0.526</td>
<td>90</td>
<td>22</td>
<td>279.8</td>
<td>72.2</td>
<td>0.475</td>
</tr>
</tbody>
</table>

Table 5
Concentration of some metabolites in blood plasma after 6 weeks of treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal (n = 6)</th>
<th>Obese (n = 6)</th>
<th>Obese receiving drug (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/ml)</td>
<td>79</td>
<td>78</td>
<td>62</td>
</tr>
<tr>
<td>Cholesterol (mg/ml)</td>
<td>74</td>
<td>77</td>
<td>74</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>0.78</td>
<td>0.87</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Fig. 2. 2D and 3D counters of RSM design for diameter of fibers.
second, it is produced by fragmentation of skin keratin so it is supposed that its similarity to skin may help drug molecules to pass through skin by creating package of macromolecules around it.

Table 2 shows statistical analysis of the build model. The Model F-value of 5.06 implies the model is significant. There is only a 4.80% chance that a “Model F-value” this large could occur due to noise.

Table 3 contains summarized contributions of each factor in properties of final fiber.

3.1. RSM optimization

Three parameters were selected and results of 17 Box-Bencken RSM are summarized in Table 4. All data were obtained by analysis of SEM images using Diameter J plug-in of Image J software [30]. Fig. 5 shows Sample of processed nanofiber SEM image with graphical result of analysis.

Table 5 shows summarized result of fiber preparation optimization using RSM. Based on results, contribution of these parameters in three responses including porosity, mean diameter and diameter standard deviation are all meaningful due to the p-value < 5%. Other models including linear of cubic did not gave significant p-value and R-square values.

Final equation for determination of diameter is:

\[
Diameter = 79.39 + 148.89B + 97.02V - 0.58 + V - 89.13 + A - 0.73 + B^2 - 1.06 + V^2 - 7663.66
\]

which A is mix ratio of dry PVA to gelatin weight, B is the percent of formic acid in gelatin solution and V is voltage as kV. Diameter is determined as nm. For standard deviation of diameter and porosity same technique was used and following equation was concluded:

\[
\text{Diameter standard deviation} = -1.55.37A + 146.08B + 49.47V
+ 7.13A + V - 0.59 + B + V - 0.73 + B^2
- 7075.82
\]

and for the porosity:

\[
\text{Porosity} = 0.201A + 2.485810B - 2.81210V - 2.542A + B
+ 1.129A + B + 0.259
\]

For selection of optimum point a few criteria were considered. First, mix ratio was maximized to prevent creation of beads. As gelatin concentration increases, reproducibility of experiment decreases which may cause by the fact that unlike PVA, molecular weight distribution and composition of gelatin varies from sample to sample. The second criteria were to minimize fiber diameter. The third goal was to reach minimum standard deviation of fiber diameter and finally maximizing porosity. Figs. 1 to 3 shows 2D and 3D result counters for each parameter.

Considering all of these criteria, an optimum point was selected for final production of patch containing drug. It is clear from Eq. (2) that standard deviation of fibers decreases as PVA to gelatin ratio increases which confirms our anticipation in experiment design. Another
advantage of adding PVA to solution is vanishing beads in fibers. But base on Eq. (1) increase in PVA increases fiber diameter. Porosity is mainly affected by voltage which is confirmed by first series of experiments for screening of factors. Increasing in voltage increases force exerted on polymer leaving needle tip in spite of what was expected based on previous study of other polymer systems [36]. Which disrupts orders of fibers landing on drum and higher porosity is obtained. This trend is observable in porosity contours (Fig. 4).

In this step, there is an optimum point for synthesis of nanofiber which can be loaded with various types of chemicals as drugs or plant extracts. Fig. 5 shows three samples synthesized based on optimum point set up. It’s observable that appearance of these three samples dose not looks to be different. After processing SEM images by software fiber radius distribution of 19 different samples produced in different times with different additives stimulating different drugs extracted which is shown in Fig. 6. These fiber radius distribution charts are normalized in such a way that integral of graph in each sample is equal to 100%.

3.2. IR characterization

In Fig. 7 IR spectrum of blank fibers and fibers containing curcumin are compared. Subtracting these to graphs leads to third line which shows the characteristic absorption band of curcumin. This shows that drug is successfully embedded inside fibers.

In FT-IR spectrum 3300 cm$^{-1}$ band is related to hydroxyl group of PVA, H bonded; 2930 cm$^{-1}$ band is for alkane C–H; 1650 cm$^{-1}$ band is stretching band for carboxyl of peptide; 1532 & 1440 cm$^{-1}$ bands are for aromatic C=C related to gelatin amino acid sidechains; 1244 & 1085 cm$^{-1}$ bands are for multiple C–O bonds probably related to alcohol C–O bonds of PVA; 1630 cm$^{-1}$ band is for stretching of ketone carboxyl groups of curcumin; 1528-aromatic C=C bonds of curcumin; 1230 & 1080-multiple C–O located on aromatic groups of curcumin;

3.3. Release profile in PBS buffer and NaCl solution

Curcumin release profile in 0.01 M PBS buffer is observable in Fig. 8. As seen in figure a burst release paradigm is observed, both for PBS and NaCl solution. But it is shown that in NaCl solution reaches to higher maximum release percent. It is because of solubility of PVA and gelatin in water which leads to fast release of curcumin-gelatin mixture in PBS buffer. This release profile is optimal because of the fact that the bottleneck of the process is drug diffusion through outer layer of skin. So increasing rate of drug diffusion from inside of nanofibers to the surface of skin should be minimized to increase total rate of delivery. Presence of gelatin in nanofiber helps to increase solubility of curcumin in water. Sweating is the source of water after attachment of fibers to skin. Release in NaCl solution investigated to simulate skin sweating in presence of transdermal patch.

3.4. Ex-vivo transdermal diffusion test

Ex-vivo transdermal release of curcumin through skin was determined using a side by side arrangement transdermal diffusion test setup, using three patch containing drug. Drug release profile of drug is
observed in Fig. 9. Based on these results, there is a delay in release which takes long for minutes and increasing profile which reaches to a steady state. Finally, this profile reaches to more than 50% of drug release after 20 h.

In comparison to other systems used for transdermal delivery of curcumin [37, 38], higher efficiency is observed in this work. Considering the skin type used for test, although goat skin [37] is thicker than rat skin, but surface area of drug delivery (0.745 cm² against 2.5 cm²) is smaller. In other work, pig ear skin is used and lower amount of delivered is effected by this higher thickness. However, high efficiency of this method for curcumin delivery can be due to higher efficiency of diffusion from fiber inside to skin surface. Another reason for this rate of transdermal diffusion may be presence of gelatin. Curcumin is a hydrophobic product in nature. So its solubility in water is very low and it is shown that its bioavailability increases dramatically when used orally [34]. In presence of gelatin, this solubility increases dramatically which makes curcumin highly available to the surface.

Replacement time of transdermal system is dependent to drug release. For drugs which enter into blood stream so fast, it should be controlled to be enough long to prevent overdose but in this case the target tissue is just below skin, besides the fact that the drug is highly hydrophobic itself and dose not tend to leave adipose tissue quickly. So fast delivery of drug would lead to lower usage time of drug patch and higher satisfaction of patient.

Fig. 5. Three samples of fibers synthesized using optimum point parameters and processed SEM in totally same condition. High repeatability of nanofiber synthesis is observed here.
3.5. Animal test

As a complementary test to prove drug effectiveness in vivo, animal test is designed. After 5 months and reaching a meaningful difference in average weight of each animal group, drug was administrated on all members of one group. 2 days after drug administration all members of groups were weighted and results were recorded. These recorded weights are shown in Fig. 10.

After 6 weeks a couple of rats from each group were subjected to be analyzed with MRI for whole body and blood test of leptin, triglyceride and cholesterol. A result of blood tests is summarized in Table 5. It has been shown previously that leptin level of blood plasma is related to

![Fig. 6. Radius of fiber distribution in 19 different samples produced based on optimum point.](image1)

![Fig. 7. IR spectrum of fibers containing curcumin (a), blank fiber (b) and subtract of them (c) which is pure curcumin.](image2)

![Fig. 8. Release profile of curcumin into PBS buffer and NaCl solution. Dash line is related to release in PBS buffer and solid line for NaCl solution.](image3)
BMI index of body [39]. By increasing body mass due to obesity, leptin level of blood increases. Based on blood test of animal’s level of leptin has decreased to even lower level of normal group in drug receiving group.

The results show that during this period of time, volume percent of adipose tissue has decreased meaningfully. It has to be kept in mind that high calorie diet is continued to be fed in this period. Figs. 11 and 12 show processed images of MRI results for an obese rat and obese rat which is administrated by transdermal patch. Use of this analysis method instead of postmortem is a non-invasive method of body composition analysis which can be used for alive animals [32]. Decrease in total adipose tissue volume of body is clearly observable in these images.

Table 6 contains data obtained by image processing of MRI results of two samples from each animal group (N, L and Dc). In each image length and width of each pixel is 0.5 and 0.5 mm respectively. These values are multiplied by distance between images so each 3D pixel is called voxel. Volume summation of all voxels is related to whole animal body volume. For volume of adipose tissue, a threshold is used to separate adipose tissue from other tissues of body. This threshold is obtained from MRI data of visually separated distinct tissues of animal’s body. This shows that volume percent of fat in drug received animals is close to normal group and is 4–7% less than obese animals.
A process from material design to animal test for a drug delivery system is presented in this work. It is shown here that use of statistical methods for design of drug delivery system from screening of parameters to optimization of production based on biocompatible polymers including gelatin and poly vinyl alcohol for entrapment of a polyphenol, curcumin, which is proven to be effective in treatment of obesity, leads to obtaining a drug delivery system which effectively delivers drug molecules through skin. Based on this method of design, fiber with radius in range of 200 to 250 nm was produced with low standard deviation. More than 19 run in the optimum point repeated and analysis of their SEM images showed that statistical distribution of radius in all of fibers was the same. It is shown here that synthesis morphology of produced transdermal patch is highly reproducible. This uniform and reproducible delivery can be a beginning to design and production of transdermal delivery system which aims elimination of adipose tissue locally in patients. Confirmation of drug delivery effectiveness is proved by monitoring total weight of animal models, blood test for level of some metabolites and MRI imaging of whole body. Leptin as a reference metabolite in studies related to obesity is shown to be in the lower level in drug receiving (0.63) group than normal group (0.78) and obese group (0.87). Whole body imaging of rats by MRI technique shows 4–7% decrease in volume percent of adipose tissue in analysis of samples from each group. Based on results it is shown that this delivery system can be easily used as a transdermal patch which effectively decrease volume of adipose tissue in obese rats. As a development for this study, design of special shape transdermal patches

### Table 6

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total voxels</th>
<th>Total fat voxels</th>
<th>Obese receiving drug (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>357,272</td>
<td>71,372</td>
<td>19.98</td>
</tr>
<tr>
<td>N2</td>
<td>343,426</td>
<td>73,525</td>
<td>21.41</td>
</tr>
<tr>
<td>L1</td>
<td>436,488</td>
<td>115,912</td>
<td>26.56</td>
</tr>
<tr>
<td>L2</td>
<td>436,900</td>
<td>98,067</td>
<td>22.45</td>
</tr>
<tr>
<td>Dc1</td>
<td>304,398</td>
<td>56,338</td>
<td>18.51</td>
</tr>
<tr>
<td>Dc2</td>
<td>340,872</td>
<td>65,800</td>
<td>19.30</td>
</tr>
</tbody>
</table>

a Total voxels of body.

b Total voxels of adipose tissue.

c Volume percent of adipose tissue in whole body obtained by division of adipose tissue voxels by total body voxels.

### 4. Conclusion

A process from material design to animal test for a drug delivery system is presented in this work. It is shown here that use of statistical methods for design of drug delivery system from screening of parameters to optimization of production based on biocompatible polymers including gelatin and poly vinyl alcohol for entrapment of a polyphenol, curcumin, which is proven to be effective in treatment of obesity, leads to obtaining a drug delivery system which effectively delivers drug molecules through skin. Based on this method of design, fiber with radius in range of 200 to 250 nm was produced with low standard deviation. More than 19 run in the optimum point repeated and analysis of their SEM images showed that statistical distribution of radius in all of fibers was the same. It is shown here that synthesis morphology of produced transdermal patch is highly reproducible. This uniform and reproducible delivery can be a beginning to design and production of transdermal delivery system which aims elimination of adipose tissue locally in patients. Confirmation of drug delivery effectiveness is proved by monitoring total weight of animal models, blood test for level of some metabolites and MRI imaging of whole body. Leptin as a reference metabolite in studies related to obesity is shown to be in the lower level in drug receiving (0.63) group than normal group (0.78) and obese group (0.87). Whole body imaging of rats by MRI technique shows 4–7% decrease in volume percent of adipose tissue in analysis of samples from each group. Based on results it is shown that this delivery system can be easily used as a transdermal patch which effectively decrease volume of adipose tissue in obese rats. As a development for this study, design of special shape transdermal patches

![Fig. 12. Whole body comparison of MRI images acquired from fat rats (A & C) and fat rats treated by transdermal patch (B & D). Red areas are adipose tissue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)](image-url)
which their shape is related to shape and volume of subcutaneous fat to can be studied. Use of magnetic resonance imaging here is a basis for determination of effectiveness of produced transdermal patch to elimination of adipose tissue locally by analysis of shape of this type of tissue before and after use of transdermal patch.

References