Eutectic Formation and Cutaneous Wound Healing by Binary Allantoin-Octadecenedioic Acid System

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Abstract

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Keywords

Allantoin; Octadecenedioic acid; Eutectic; Wound healing

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Authors

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Abstract

Eutectic formation in allantoin-octadecenedioic acid system (AOM) has been studied. The binary phase diagram revealed the existence of eutectic at $X_{allantoin} = 0.7$. Dioic acid caused disruption of network in allantoin and induced defects, ultimately causing the reduction in crystallinity as shown by XRD and SEM analysis. The thermal stability in TGA graphs of allantoin was negligibly altered after mixture formation. Classical hydrogen bonding and alkyl interactions were predicted using software Avogadro and BIOVIA Discovery Studio. The functionalities involved in interactions taking place in the system were studied through in-depth FT-IR spectroscopy. The eutectic showed efficacy for cutaneous wound healing in oryctolagus cuniculus domesticus, hence it could be used as therapeutic for regulation and control of bioactivity.

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1. Introduction

Wound healing involves several complex processes occurring in a highly regulated way. In the absence of regulation cancer is likely [1]. Hence any wound healing intervention would ensure control beyond potency [2]. Allantoin promotes the healing process through maintaining hydration levels, stimulation of epithelization and facilitation of keratolytic action. Moreover, it increases cell mitosis, eliminates necrotic tissues and has analgesic effect [3,4]. Allantoin has affinity for aqueous phase as reflected by is negative logP (i.e., $-2.89$ ChemSketch/ACD). It is also not very stable at basic pH observed at the wound site [5]. The first report on formation of eutectics for the purpose of wound healing was published in 2019 [6]. However, eutectics based on allantoin have never been reported, and emphasis has been laid mostly on preparation of (bio)materials including allantoin [7–9]. Recently, hydrophobic deep eutectic solvents based on fatty acids have been tested for enhancing the bioavailability of anti-inflammatory agent [10]. (9E)-octadecene-1,18-dioic acid is known for antiaging and skin whitening agent [11]. Its biological potential includes the activation of peroxisome proliferator-activated receptor gamma (PPAR-γ), the deficiency of which causes delay in healing of wounds [12]. Additionally, it may act as an efficient permeation enhancer in the form of eutectic for delivery of allantoin into desirable depth [13]. Deep eutectic may act as vehicles for drug delivery, controlled or sustained release or act as therapeutic itself, if at least one of the components of the mixture is bioactive [14–17]. The eutectic formation is facile and its properties can be tuned merely by changing the proportion of the constituents [18]. The importance of deep eutectic mixtures as therapeutics may be reflected in these words from a recently appeared review [19].
What if a new technology based on therapeutic deep eutectic systems would disrupt the current treatment of major economic and socially burden diseases? 

The present work deals with preparation and investigation of eutectic phenomenon in allantoin-octadecenedioic acid, and its characterization via FT-IR spectroscopy, X-ray diffraction, Scanning electron microscopy and thermogravimetric analysis. The biological potential of eutectic in wound healing was studied in domestic rabbit models. The use of in silico modeling/docking tools allow the portrayal of binding behavior and association taking place in the system. The restricted mobility of constituents in eutectics and competing interactions are expected to regulate release in these systems. Hence, the release of excess quantities of drug and its detrimental consequences can be avoided.

The self-regulation and control possible in therapeutic eutectics may lead to development of minimum or no-waste formulations, as no additional controlling agent is required. This would significantly impact the effectiveness of treatment and the economy of health care.

2. Material and methods

2.1. Material

Allantoin (≥98%) was purchased from Sigma-Aldrich. High purity trans 9-octadecene-1,18-dioic acid and pola wax were acquired from Cosmetic Pharmacy (Pakistan).

2.2. Preparation of allantoin-octadecenedioic acid mixtures (AOM)

Nine binary mixtures between allantoin and octadecenedioic were prepared (codes AOM1-AOM9), where mole fraction of allantoin was varied from 0.1 to 0.9. For each preparation, first octadecenedioic acid was melted in a fixed quantity in a precleaned and thoroughly dried glass vial. To this a desired quantity of allantoin was added and heating was continued till liquefaction. The heating of homogeneous mixture was continued at liquefaction temperature for ~1 h to ensure completion of the process.

2.3. Characterization of allantoin-octadecenedioic acid mixtures

The melting points of pure components and their corresponding mixtures were recorded using Galenkamp melting point apparatus. FT-IR spectra of selected AOM were recorded with Varian/Digilab FTS7000 spectrometer using KBr disc. The thermogravimetric analysis was carried out in a TA Instruments Q600 V20.9 using nitrogen atmosphere. The heating rate was 20 °C/min. XRD studies were performed with X-ray diffractometer D8 Advanced Davinci (Bruker), and microscopy images were taken using SEM-MIRA3 TESCAN. XRD data was processed using Xpert Highscore Plus (v 3.0).

2.4. In silico molecular modeling and docking

Avogadro and BIOVIA Discovery Studio were harnessed for molecular modeling. The geometry optimization took place automatically in Avogadro. The default all-atom universal force field (UFF) was utilized for simulations of molecular mechanics. The calculations were performed using a five-molecule system consisting of allantoin and dioic acid in the ratio 3:2 under no constraints. The energy minimization involved steepest descent that worked very well close to true global minimum. Molecular docking was performed using an online server (https://mcule.com/apps/1-click-docking/) to highlight association between PPAR-γ and octadecenedioic acid. This server utilizes autodock vina to reveal features of ligand-receptor binding.

2.5. Wound healing potential

All wound healing procedures were performed in accordance with international/national guidelines. Ethical approval was obtained from Institutional Review Committee in this regard. Six rabbits each weighing between 1.5 and 2.0 kg were divided into two groups. One rabbit was treated with placebo, while others were treated with ointment possessing different percentages of eutectic. Local anesthesia was administered using 4 mg of xylazine plus 40 mg ketamine hydrochloride per kg of body weight. Each cutaneous incision (1 cm) was made on the shaven dorsa of rabbits using scalpel. The ointment (A-E) containing 5–25% AOM in pola wax was applied to wound area once in 24 h for 10 days. The images were processed using ImageJ software (NIH).

3. Results and discussion

3.1. Solid-liquid binary phase diagram

Fig. 1 shows the solid–liquid phase diagram of allantoin and octadecenedioic acid system (AOM). The gradual melting point depression occurs with an increase in proportion of allantoin. At eutectic
point \( (X_1 = 0.7) \) \( \Delta T = 144 \) °C with reference to melting point of pure allantoin. It is apparent from the plot that the network existing in pure allantoin (with ureidyl groups forming a channel) is disrupted in the presence of dioic acid [7]. Though the lowest melting point is exhibited by mixture consisting of dioic acid:allantoin in the ratio 3:7, the difference between the melting points of mixtures having \( X_1 \) in the range 0.3–0.7 is merely 1–3 °C.

The results obtained using predictive software are shown in Fig. 2 for AOM having \( X_1 = 0.6 \). Apart from conventional hydrogen bonding, hydrophobic interactions are seen that involve the alkyl backbone of acid.

### 3.2. X-ray powder diffraction, thermogravimetry and scanning electron microscopy

To further investigate the effect of dioic acid presence on order in allantoin, X-Ray powder diffraction analysis was carried out and the plots of selected mixtures are gathered in Fig. 3. Allantoin in pure form is monoclinic and does not exhibit polymorphism [28]. In AOM, crystallinity is reduced due to presence of dioic acid. The crystallite size calculated for AOM2, AOM5 and AOM8 were 177 Å, 302 Å and 357 Å, respectively. The corresponding dislocation density values were \( 3.19 \times 10^{-5} \) Å\(^{-2} \), \( 1.09 \times 10^{-5} \) Å\(^{-2} \), and \( 7.86 \times 10^{-6} \) Å\(^{-2} \), which show the extent of defects in the sample [29]. The carbonyl groups in allantoin act as hydrogen bond acceptors, while \( \text{NH}_2 \) and \( \text{NH} \) functionalities act as hydrogen bond donors. This leads to complex template with ureido moieties forming a channel [7]. This complex array is broken down upon dioic acid addition. Hence, the order existing in pure allantoin is disrupted when eutectic is formed, and association is developed between different functionalities in allantoin and carboxyl groups of dioic acid. The presence of excess acid triggers self-association between dioic acid molecules also consequently, the order in the system is further changed.

Thermal degradation plots (Fig. 4) show that AOM5 and AOM8 are only slightly more stable thermally than AOM2. All the mixtures were nearly as stable as allantoin [30]. SEM images gathered in Fig. 5 also endorse our earlier observation based on XRD analysis. AOM8 appears more crystalline, whereas gel like structure prevails in the presence of higher proportion of dioic acid. An analysis of SEM images shows that the crystal surface has inhomogeneities and there exist a few voids, however,
the edges are not sharp, and voids seem partly filled with eutectic. The structure is layered, with retention of network in some regions [31]. With increase in proportion of allantoin, layered surface becomes more homogeneous, where pores have been filled to significant extent due to presence of eutectic. However, some cracks are still obvious [32]. The absence of cracks reflects solidification from melt [33].

3.3. Fourier transform infrared spectroscopy

Region I: 3500–2800 cm$^{-1}$

Table 1 shows infrared vibrational frequencies of pure allantoin, octadecenedioic acid and their mixtures AOM2, AOM5 and AOM8. The labelled allantoin molecule is provided in Fig. 6. Octadecenedioic acid absorbs at 3500 cm$^{-1}$ due to O–H stretching [34]. The peak at 3425 cm$^{-1}$ is due to $\nu_{as}$ (NH$_2$) of ureidyl moiety. AOM shows frequencies in-between these values, indicating the engagement of both OH and NH$_2$ in H-bonding interactions. The frequencies exhibited by AOM5 and AOM8 were 3437 cm$^{-1}$ and 3436 cm$^{-1}$, respectively. The corresponding symmetric stretching vibrations appear at 3340 cm$^{-1}$ in spectra of the mixtures, which is similar to that in pure allantoin [35]. Either of the vibrations involving NH$_2$ are not visible in AOM2 probably due to presence of low quantity of allantoin ($X_1 = 0.2$) in the mixture. Also, vibration at 3531 cm$^{-1}$ is obscured for AOM2. The shifting of NH$_2$ stretching vibrations to higher frequency shows weakening in strength and decrease in number of hydrogen bonds [36,37]. This is possibly due to disruption of hydrogen bonding network in allantoin in the presence of dioic acid. $\nu$(N3–H3) with frequency 3190 cm$^{-1}$ in pure allantoin appeared at 3192 cm$^{-1}$ and 3191 cm$^{-1}$ with $X_1 = 0.5$ and 0.8, but not seen in the spectrum with $X_1 = 0.2$. These values are not changed and show the absence of interactions involving N–H of the ureidyl group. The C–H stretch of sp$^2$ hybridized carbon atom of C=C has frequency 3034 cm$^{-1}$ in dioic acid [34]. These values are increasingly shifted to higher wavenumber with increase in proportion of allantoin from 3035 cm$^{-1}$ in AOM2 to 3050 cm$^{-1}$ in AOM8 revealing the existence of non-covalent bonding, which is different from that in pure acid. The values for antisymmetric and symmetric stretching vibrations due to CH$_2$ of alkyl groups are around 2920 cm$^{-1}$ and 2850 cm$^{-1}$ in pure acid as well as mixtures, and a slight deviation of 2–3 cm$^{-1}$ to lower wavenumber is seen in mixtures compared to pure acid. The change may be significant being representative of the order in the alkyl chain packing [38].

Region II: 1800-1250 cm$^{-1}$

In this region, the absorptions due to allantoin include $\nu_{as}$ [(C1=O1) + (C2=O2)], $\nu$(C3=O3), $\beta_{scis}$ (NH$_2$), and C–N stretching, which appear at...
Table 1. FT-IR data of selected allantoin-octadecenedioic acid mixtures and corresponding pure constituents.

<table>
<thead>
<tr>
<th>AOM2</th>
<th>AOM5</th>
<th>AOM8</th>
<th>Allantoin</th>
<th>Assignment</th>
<th>Octadenedioic Acid</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>3436</td>
<td>3437</td>
<td>3425</td>
<td>νas (NH$_3$)</td>
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<td>ν(OH)</td>
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<td>–</td>
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<td>3340</td>
<td>3340</td>
<td>νs (NH$_2$)</td>
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<td>–</td>
<td>3192</td>
<td>3195</td>
<td>3190</td>
<td>ν(ν3–H3)</td>
<td>3034</td>
<td>ν (CH)</td>
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<td></td>
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<td>1779</td>
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<td>1799</td>
<td>1740</td>
<td>νas [(C1=O1) + (C2=O2)]</td>
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<td>1602</td>
<td>–</td>
<td>1603</td>
<td>β$_{scis}$ (NH$_3$)</td>
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<td>δ(OH)</td>
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<tr>
<td>1400</td>
<td>1399</td>
<td>1401</td>
<td>1430</td>
<td>β[H2–N2–C2 + H5–C5–C2]</td>
<td>1184</td>
<td>ω(CH$_3$)</td>
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<td>1319</td>
<td>1320</td>
<td>1324</td>
<td>1326</td>
<td>β[H2–N2–C2 + H5–C5–C2] and ν (hydantoin ring)</td>
<td>1083</td>
<td>μ(CH$_3$)</td>
</tr>
<tr>
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<td>1183</td>
<td></td>
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<tr>
<td>819</td>
<td>817</td>
<td>815</td>
<td>860–510</td>
<td>ν(N–H)</td>
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<td></td>
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<tr>
<td>767</td>
<td>760</td>
<td>760</td>
<td>780–760</td>
<td>ν(C=O)</td>
<td></td>
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</tr>
</tbody>
</table>

1740 cm$^{-1}$, 1680 cm$^{-1}$, 1603 cm$^{-1}$, and 1400 cm$^{-1}$, respectively [35]. The asymmetric stretching frequency of C=O of the hydantoin ring is shifted to 1779 cm$^{-1}$ in AOM. The value 1701 cm$^{-1}$ representing C=O of dioic acid is shifted to 1689 cm$^{-1}$, 1688 cm$^{-1}$ and 1690 cm$^{-1}$ in AOM2, AOM5 and AOM8. The ureido carbonyl is nearly 25 cm$^{-1}$ shifted to lower wavelength. β$_{scis}$ (NH$_3$) is negligibly altered after mixture formation. This shows involvement of different types of carbonyl groups in hydrogen bonding interactions to different extents. The C–N stretching frequency of allantoin on average moves to about 10 cm$^{-1}$ towards higher wavenumber region in mixtures. The in-plane bending vibrations β[H3–N3–C + H5–C5–N3] as well as stretching ν[C3–N1–C5] is seen ~ 1530 cm$^{-1}$ in pure allantoin [35,39]. These frequencies are red shifted (Δν = 5–7 cm$^{-1}$) in AOM depending upon the mole fraction of allantoin. The O–H in-plane bending vibrations (1472 cm$^{-1}$ in pure acid) are also red shifted by 1–2 cm$^{-1}$ in the mixtures. β[H2–N2–C2 + H5–C5–C2] combined with stretching frequency of five membered ring is centered at 1326 cm$^{-1}$ [35]. This value is regained from 1319 cm$^{-1}$ in AOM2 to 1320 cm$^{-1}$ and 1324 cm$^{-1}$ in AOM5 and AOM8. β[H4–N4–C3] + β[H5–C5–N3 + H14–N–C] + νas (N–C3) + β[O3=C3–N] is present at 1284 cm$^{-1}$ [35]. The C–O stretching frequency of dioic acid also appears around this value [40]. The vibrational frequency of this band was 1294 cm$^{-1}$, 1291 cm$^{-1}$ and 1282 cm$^{-1}$ in AOM2, AOM5 and AOM8, respectively. These changes indicate that intermolecular hydrogen bonding is severed [41].

Region III: 1200–450 cm$^{-1}$

Allantoin shows peak at 1184 cm$^{-1}$ due to cumulative ν(N3–C5), stretching frequency of five membered heterocycle and bending β[H5–C5–C2]. In addition, ν(N3–C5) gives peak at 1136 cm$^{-1}$ [35]. AOM2, AOM5 and AOM8 show peaks at 1192 cm$^{-1}$, 1191 cm$^{-1}$ and 1183 cm$^{-1}$. However, peak at 1137 cm$^{-1}$ is only observed with AOM8 having high allantoin content. It is worthwhile to note that CH$_2$ wagging also appears at 1190 cm$^{-1}$, causing the overlapping of vibrational modes in allantoin and

![Fig. 6. Allantoin molecule with atom descriptors.](image)
dioic acid. They represent involvement of these groups in interactions [36,37]. ν(C–NH₂) of allantoin produces a low intensity band in the region 1120–910 cm⁻¹. N–H out-of-plane bending appears between 860 and 510 cm⁻¹ and additionally at 450 cm⁻¹. The C=O vibration is also observed in the range 780–760 cm⁻¹ [39]. In dioic acid the frequency at 962 cm⁻¹ that is clearly visible in all mixtures shows the presence of trans double bond [34]. The change in these frequencies reflects the modulation of hydrogen bonding [36].

3.4. Wound healing potential

Wound healing in rabbit models was monitored for ten days after application of ointment containing different percentages of AOM5. The corresponding images are gathered in Fig. 7. All wounds treated with AOM5 were healed on the 6th day. However, maximum wound contraction was observed with D, containing 20% of the active ingredient. In comparison, negligible contraction was seen in the case of F, treated with placebo.

It is worthwhile to note that the eutectic may release active ingredients because of competitive interactions or microenvironment at the wound site. Hence, release is regulated. Besides the release of allantoin, the healing effect may be related to interaction between Peroxisome Proliferator-Activated Receptor (PPAR) and dioic acid [42]. The energetically most favorable association between PPAR-γ and octadecenedioic acid as revealed by 1-click docking is shown in Fig. 8.

4. Conclusion

A novel allantoin-octadecenedioic acid eutectic is reported. The eutectic point was observed at 86 ℃ with mixture containing 70 mol percent of allantoin. This represents a differential melting point depression of 144 ℃ compared to pure allantoin. This shows that the presence of dioic acid significantly changes order in allantoin and intermolecular hydrogen bonding between allantoin molecules is severed. The defects or dislocations increase with increase in content of dioic acid probably due to self-association between dioic acid molecules. The use of in silico modeling tools indicates the existence of classical hydrogen bonding and alkyl interactions. However, the thermal stability of allantoin is not affected after eutectic formation. As indicated by SEM image analysis, voids in surface are filled upon eutectic formation. Eutectic was effective in healing cutaneous wounds in domestic rabbits. Though AOM shows comparable acceleration of healing of wounds, maximum contraction is seen with concoction containing 20% active ingredient. Here, allantoin’s efficacy is enhanced through activation of peroxisome proliferator-activated receptor gamma by dioic acid. This may also be due to greater permeability of allantoin in the presence of dioic acid.
Conflict of interest

The author(s) declare(s) that they have no competing interests.

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References


