PHOTODYNAMIC ANTIMICROBIAL CHEMOTHERAPY OF WATER SOLUBLE CURCUMIN POLYMERIC PRODRUG

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ABSTRACT
Curcumin has diferuloylmethane nucleus effective in treating bacterial and fungal manifestation. Poor solubility and reduced systemic bioavailability compromises its therapeutic potential. To circumvent this problem we have chemically conjugated curcumin to biodegradable and bioadhesive poly (methylvinyl ether/maleic anhydride) via ester linkage. The amphiphilic nature of polymeric prodrug of curcumin has exhibited improved aqueous solubility of 14.54 gm/L, 53 times higher compared to pure curcumin, hydrolytic stability at pH 7.4 for 7 hrs with bioadhesive potential. The photodynamic therapy potential of conjugate evaluated at 460nm at 50J/cm² performed invitro against microbial strains-gram negative E. coli. ATCC 25922, gram positive S. aureus ATCC 2654 and Candida albicans ATCC 2091 revealed 50% increase in antimicrobial activity compared to the native drug. The product has demonstrated remarkable antimicrobial activity and can have potential use in skin, oral and dental antimicrobial applications.
INTRODUCTION

Curcumin is a polyphenol\cite{1} with distinctive properties, unique molecular architecture and multipotent efficacies. Its antiseptic activity has been known since ages. It is taken orally for immunomodulatory effects and topically applied on wounds\cite{2} for its healing properties. Diferuloylmethane nucleus has an effective antibacterial and antifungal activity\cite{3}. It is a hydrophobic molecule belonging to BCS class IV that exhibits low aqueous solubility and poor bioavailability\cite{4,5}. In addition, it undergoes rapid metabolism in vivo to form glucuronide conjugates\cite{6}. Thus, large doses of curcumin are required to be administered for the desired therapeutic effects to be manifested\cite{5}. This crucial issue can be resolved by enhancing the solubility profile of curcumin in water\cite{7} and manipulating its partition coefficient. The macromolecule prodrug application of curcumin could play a role in augmenting its aqueous solubility,\cite{8} controlled release profile\cite{9} and bioavailability.\cite{10}

Maleic anhydride copolymers have been used extensively in novel polymer prodrug delivery. Chemical conjugation of styrene-maleic anhydride copolymers with gemfibrozil,\cite{11} fenoprofen,\cite{11} ibuprofen, paracetamol\cite{12} and doxorubicin\cite{13} to improve aqueous solubility have been reported. Poly (methylvinyl ether/maleic anhydride) marketed as Gantrez\textsuperscript{®} AN marketed by ISP has been chemically cross-linked with serum albumin and gelatin for
preparing bioadhesive nanoparticles.\textsuperscript{14} Gantrez\textsuperscript{®} AN 119 is a synthetic bioadhesive copolymer with wide pharmaceutical applications such as denture adhesive, transdermal patches, mucoadhesive drug delivery, thickening and suspending agent.

Cur-PMEM, polymeric conjugate of Gantrez\textsuperscript{®} AN 119 and curcumin was prepared by simple esterification technique and characterized by FTIR, XRD and UV spectroscopy. Mucoadhesive strength, hydrolytic stability and solubility in various solvents were evaluated with different grafting ratios of curcumin onto the polymer. The ester linkage served as the hydrolytically and enzymatically cleavable bridge between the drug and polymer. Curcumin has manifested antimicrobial effects clinically\textsuperscript{15} in the presence of photodynamic therapy.\textsuperscript{16,17} Antimicrobial evaluation using a whole cell assay was performed to evaluate the minimum inhibitory concentration of native drug along with its polymeric prodrug in presence and absence of photons. The study revealed dual advantage of conjugation and photodynamic therapy in terms of therapeutic response against \textit{E.coli}, \textit{S. aureus} and \textit{candida albicans}. The research work aimed at the chemical modification of curcumin with Gantrez AN 119 to form tailor made macromolecular prodrug with photodynamic activity to treat topical and oral infections.

\textbf{MATERIALS AND METHODS}

\textbf{Materials}

Poly(methylvinyl ether/maleic anhydride) copolymer (Gantrez\textsuperscript{®} AN 119) was kindly gifted by ISP, Mumbai, India. Curcumin (95\% (w/w) was a gift sample from Konark Pharmaceuticals, Mumbai, India. Concentrated Hydrochloric acid (Analytical Reagent Grade) and Acetone (Analytical Grade) were procured from Spectrochem, Mumbai.

All commercially available solvents and chemicals were utilized without further purification. Water used throughout the study was distilled water (Density = 0.997gm/ml). Dialysis membrane (70 LA393-10MT) made of regenerated seamless cellulose tubing was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India.
Methods

Synthesis of Curcumin-graft-poly(methylvinyl ether/maleic acid) (Cur-PMEM) conjugate

Poly(methylvinyl ether/maleic anhydride) copolymer (molecular weight 200 kDa) was dissolved in acetone at room temperature and heated up to 80 °C in a condenser equipped reaction vessel. The chemical reaction is described in Figure 1. Curcumin (molecular weight 368.38 Da), previously dissolved in acetone was introduced to polymer vessel. The reaction mixture with varying moles of reactants (Table 1) was acidified using few drops of hydrochloric acid (pH 1-2) and stirred continuously at 80 °C for 24 h. The reaction was monitored qualitatively using silica gel thin layer chromatography plates and Infrared spectroscopy. Resultant mass was cooled down to room temperature and subjected to dialysis in a dialysis membrane (MWCO 14,000) against acetone for 1 day and then against distilled water for 2 days. Acetone and distilled water were changed periodically to avoid saturation. Product was subsequently freeze-dried (FreezoneLyophilizer, Labconco, USA). Fluffy material was crushed in dehumidified conditions to obtain a dry, orange powder. The grafted polymer was stored at room temperature in an amber glass vial until further analysis.

Characterization (Cur-PMEM) conjugate

Fourier Transformer Infrared Attenuated Total Reflection (FTIR-ATR) spectroscopy (FTIR)

The infrared absorption spectra of Poly(methylvinyl ether/maleic anhydride), Cur-PMEM and Curcumin were determined using FTIR spectrophotometer (FT-IR Spectrum RX 1, Perkin
Elmer) in the range of 4000-400 cm\(^{-1}\). The FTIR spectras were recorded in potassium bromide discs with 16 scans.

**Determination of Curcumin loading content and loading efficiency**

The curcumin loading content and loading efficiency in different Cur-PMEM conjugates was determined by UV-VIS spectrophotometer (Shimadzu 1650) at 420nm. The absorbance range of Cur-PMEM conjugates was read against the reagent blank by using a UV-Vis.\(^{[19]}\) Free curcumin in dimethylsulfoxide (DMSO) was used to prepare a standard concentration curve for the estimation of curcumin content in Cur-PMEM conjugates. Samples were dissolved in DMSO by dissolving varied amounts of curcumin and curcumin conjugate. The concentration of curcumin in the conjugate was estimated from the absorption intensity of the conjugate in the standard calibration curve. The absorbance maxima of Cur-PMEM conjugates was also measured.

\[
\text{Loading content} = \frac{\text{Amount of curcumin conjugated to polymer}}{\text{Amount of polymer weighed initially}} \times 100
\]

\[
\text{Drug Loading efficiency} = \frac{\text{Amount of curcumin conjugated to polymer}}{\text{Amount of curcumin weighed initially}} \times 100
\]

**Solubility**

Solubility of the conjugates was identified in different aqueous and organic solvents as per modified technique reported by Manju and Sreenivasan.\(^{[8]}\) Solubility of curcumin and Curcumin-graft-poly(methylvinyl ether/maleic acid) conjugates was determined by adding an excess amount of curcumin and Cur-PMEM conjugates separately to an aqueous phosphate buffer saline (pH 7.4). The solutions were vortexed for 5 min, sonicated for 10 min, and centrifuged at 20,379 g for 5 min. The supernatant was diluted with DMSO. The concentration was read from the standard curve of pure curcumin as described above.

**X-Ray Diffraction/Crystallinity**

X-ray diffraction graph of Cur-PMEM conjugates was recorded on Rigaku Miniflex XRD instrument (Japan). The X-rays were generated from CuK\(\alpha\) of 1.54 A\(^{\circ}\) intensity. The analyte was placed in sample holder and scanned through angle 2 to 60\(^{\circ}\) at the speed of 0.02\(^{\circ}\) /min, step size of 0.0170 and scan step time of 10s.
Mucoadhesion evaluation

The mucoadhesive strength of Cur-PMEM was determined by a double beam physical balance apparatus\[20,21\]. Freshly excised intestinal mucosa of rat was procured from Haffkine Biopharmaceutical Research Institute, Parel, Mumbai. The animal study protocol was approved by the Animal Ethics committee ICT/IAEC/0911/34. CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines adhered during the entire maintenance and experimental period. The mucosal tissue was washed with deionized water to remove non-digested food from lumen and then placed in freshly prepared saline solution\[22\]. It was tied to the Teflon block with the mucosal side exposed, following which the Teflon block was attached to the left hand beam of the balance. The mucosal tissue was kept in contact with the phosphate buffer saline (PBS) solution pH 7.4 at 37±1 °C such that the fluid just reached the surface of the mucosal tissue and kept it moist. For measuring the force of mucoadhesion, 100 µl of 10% solution of Cur-PMEM conjugates in water were placed on a glass plate and was brought in contact with the mucosa. After a contact time of 3 min, pre-calibrated weights were gradually added to the pan on the right side of the balance until the Teflon block bearing the mucosa detached itself from the polymer. The mucoadhesive strength was calculated in ‘mg’. The test was performed in triplicates.

Hydrolytic stability study of GCU11

Hydrolytic stability of representative Cur-PMEM conjugate GCU 11 was determined. 10mg GCU11 was dissolved in 10 mL PBS buffer pH 7.4. Absorbance of the solution was measured at 420nm using UV-Vis spectrophotometer. The absorbance was read from 0 to 7 h with time interval of 1 h. Graph of % decay of curcumin versus time was plotted to study hydrolytic stability of curcumin in GCU11 at physiological pH.

Nanoparticle preparation

Nanoparticles of G-CU 11 were prepared by solvent evaporation technique. A solution of 10 mg conjugate dissolved in acetone (5 mL) was added dropwise in aqueous solution (10 mL) containing 0.1% tween 80 with continuous stirring on a magnetic stirrer for 30 min at room temperature. The organic phase was evaporated under vaccum (Labrota, Heidolf, Germany). The remaining aqueous solution was freeze-dried to obtain light orange colored nanoparticles.
Surface morphology and size of nanoparticle

Surface morphology of nanoparticle was photographed under TEM (CM 200 Philips Inc. Briarcliff Manor NY USA). Nanoparticle dispersion with 0.1% phosphotungstic acid (1:1 v/v) was dropped over copper grids and left for 15 min in contact. Grids were air-dried for 24 and then analyzed with TEM. Particle size was determined by photon correlation spectroscopy (PCS; Beckman Coulter N4 plus, Wipro, India). Suitably diluted (10x; double distilled water) and filtered (0.45μm membrane filters; Pall Life sciences, Mumbai, India) samples were analyzed at an angle of 90° at 25°C. Each sample was analyzed in triplicate, and average particle size and polydispersity index (PI) were measured.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetric measurements of Poly(methylvinyl ether/maleic anhydride), GCU-11 and Curcumin were performed on Pyris 6-DSC equipment, Perkin Elmer over a heating range of 40-250 ⁰C at a heating rate of 10 ⁰C/min. Lyophilized samples were placed in a aluminium pan and crimped. Nitrogen gas was purged through samples at the flow rate of 20mL/min. The data obtained was processed by Pyris software.

Anti-microbial whole cell assay

Microbroth dilution method was used for anti-microbial testing based on the M27-A broth microdilution reference procedure of the National Committee for Clinical Laboratory Standards (NCCLS).[23] The testing was carried out in sterile microdilution plates with 96 flat bottomed wells (HiMedia Laboratories Pvt. Ltd.). Stock solutions (10mg/mL) of Cur-PMEM conjugate, poly(methylvinyl ether/maleic anhydride) and curcumin were prepared in dimethyl sulfoxide. Further dilutions were prepared with broth medium (Sigma Chemical) (pH 7.2-7.4). The culture inoculum was adjusted to a concentration of 10⁵ CFU/ml, and an aliquot of 100 µl was added to each well of the microdilution plate. Negative and positive controls were maintained in triplicates. The positive control composed of 100 µl of broth medium and 100 µl of the inoculum solution. The negative control composed of 200 µl of broth medium. The plates were incubated at 37°C for 24h on a shaker. Resazurin dye was used to determine the antimicrobial activity.[24] The lowest concentration that resulted in a significant inhibition of the growth of microbes compared to positive control was identified as the minimum inhibitory concentration (MIC).[25] Experiments were performed in triplicates to calculate the MIC value.
Photodynamic assay
A photodynamic assay was carried out on Candida albicans, Steptococcus aureus and Escherichia coli for curcumin and its synthetic polymeric conjugates. The absorption spectrum of curcumin indicated the optimal wavelength for illumination in the blue region of the spectrum (~460nm).\(^{[26]}\) Prior to the photodynamic therapy (PDT) experiment, a light emitting diode (LED), illuminating in the blue region was selected and its wavelength was measured using Constant Deviation Spectrometer Make: Besto. The intensity of light falling on the 96-well plate for the duration of 30min\(^{[26]}\) was identified using a lux meter. The LED was projected on the lux meter at different distances keeping time constant. A graph of intensity against square of distance was plotted. The slope gave the value of a constant which was utilized to measure the distance at which the plate received 50J/cm\(^2\) light fluence. The standard suspensions of microbes were photosensitized with curcumin, GCU 11 and Gantrez AN 119 and exposed to LED(P+L+) for 30 min. Group with pure curcumin and conjugate were added to wells without the LED was referred as (P+L-)\(^{[27]}\). Control group with no drug and only light was called (P-L+). Negative control group composed of cells not exposed to LED light or curcumin was called (P-L-). Serial dilutions were obtained and plated in triplicate. Plates were incubated at 37°C for 24h for E. coli and S.aureus; and 48h for C.albicans, and then read by ELISA (Awareness Technology Inc.) and the MIC of drug in native and its conjugated form were calculated. The results are combined with antimicrobial whole cell data and interpreted in results and discussion.

RESULTS AND DISCUSSION
Synthesis and purification of Cur-PMEM
The synthetic scheme involved esterification reaction between anhydride of polymer and phenol of curcumin in acidic conditions and high temperature. Nucleophilic -OH attacks cyclic anhydride of polymer, opens up the ring generating an ester and carboxylic acid group. Curcumin, polymer and the conjugate were soluble in acetone. Curcumin is insoluble in water whereas PMEM is susceptible to hydrolysis in aqueous region preventing ester linkage between drug and polymer. Hence acetone proved to be a good solvent for the reaction. Moreover, in acidic media, curcumin acts as a good proton donor. The resultant polymeric prodrug was purified by dialysis against acetone to remove unbound curcumin, and later with distilled water to convert unreacted anhydride of copolymer to carboxylic acid. It was further subjected to freeze drying to obtain fluffy orange product which was crushed into a dry powder under dehumidified conditions. The molar ratios of anhydride of PMEM to drug for
conjugation were varied from 2.5:1, 5:1, 10:1 and 15:1 as described in Table 1. The reaction achieved 61.95%, 31%, 13.4% and 9% drug conjugation respectively. The conjugation appeared approximately in multiples of its reacting ratios. Molar ratio of 1:1 was not tried since achieving 100% conjugation with a large size curcumin molecule was not practical due to the steric hindrance and spatial limitations.

Table 1: Composition and parameters of Curcumin grafted copolymer compounds. All values expressed as means ±SD. All experiments performed in triplicates.

<table>
<thead>
<tr>
<th>Curcumin grafted copolymer code</th>
<th>Moles of anhydride of copolymer equivalent to 1 mole of curcumin</th>
<th>Loading content (%)</th>
<th>Loading efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CU 11</td>
<td>2.5</td>
<td>61.95±2.83</td>
<td>65.61</td>
</tr>
<tr>
<td>G-CU 15</td>
<td>5</td>
<td>31±2.4</td>
<td>65.65</td>
</tr>
<tr>
<td>G-CU 17</td>
<td>10</td>
<td>13.4±0.9</td>
<td>57.4</td>
</tr>
<tr>
<td>G-CU 18</td>
<td>15</td>
<td>9±1.2</td>
<td>57.17</td>
</tr>
</tbody>
</table>

Structural characterization

Curcumin in DMSO exhibits an intense absorption peak at 420 nm in the UV-Vis spectral region. PMEM did not have a chromophore in the structure, and was hence, not detected by Ultraviolet spectroscopy. The Cur-PMEM conjugate dissolved in DMSO showed an absorption maximum at 434 nm in the UV region due to presence of curcumin in conjugate. However presence of auxochromic groups (ester linkage) led to red shift\[8\] confirming a change in chemical structure of curcumin and polymer.

The FTIR spectra (Figure 2, 3 and 4) of Curcumin, Cur-PMEM and PMEM showed different absorption patterns. The disappearance of the anhydride absorption band regions (1779.5 cm\(^{-1}\) and 1856 cm\(^{-1}\)) and the appearance of acid C=O (1708 cm\(^{-1}\)), ester C=O (1729 cm\(^{-1}\)), C-O (1200 cm\(^{-1}\)) and the aromatic band (3030 cm\(^{-1}\)) region in the spectra of Cur-PMEM further confirmed the grafting of curcumin on the polymer backbone. Figure 2 shows a comparative FTIR spectra of admixture of PMEM and curcumin (5:1) and Cur-PMEM conjugates. The admixture clearly showed functional groups of both curcumin and the original polymer whereas the grafted polymer showed different functionalities. Figure 3 represents FTIR spectra of G-CU11, G-CU 15, G-CU 17 and G-CU 18 polymeric drug conjugates. All the conjugates showed ester bond formation but varied in % transmittance.
Figure 2: FTIR spectra of Curcumin and poly(methyl vinyl ether/ maleic anhydride) copolymer.

Figure 3: FTIR spectra of a) admixture of poly (methyl vinyl ether/ maleic anhydride) copolymer and curcumin (5:1) and b) Curcumin-graft-poly(methyl vinyl ether/ maleic acid) copolymer molar ratio 5:1.
Figure 4 The FTIR spectra of G-CU11, G-CU 15, G-CU 17 and G-CU 18 Curcumin-graft-poly(methyl vinyl ether/ maleic acid) conjugates

X-Ray Diffraction (XRD) studies

Figure 4: XRD graph of Curcumin-graft-poly(methylvinyl ether/maleic acid).
The polymeric nature of Cur-PMEM was studied using wide angle x-ray diffraction (WAXD) technique. The sharp peaks at 43.71, 50.9889 and 75.74°2Th represented signature of a face centred cubic (FCC) crystal lattice of polymeric prodrug of curcumin which is very different from characteristic peaks of curcumin (Fig 4). The XRD pattern of poly(methylvinyl ether/maleic anhydride) shows broad peak from 22-25 theta representing the amorphous nature of polymer. Multiple sharp peaks of crystalline curcumin are observed from 10-30°2Th. All the conjugates have characteristic peak different from original reactants giving a proof of conjugation and change in structural property.

**Solubility profile**

Poor aqueous solubility of curcumin is major limitation in curcumin therapeutics. Chemical conjugation to hydrophilic polymeric backbone has resulted in amphiphilic property with dual advantage of drug and polymer properties. Table 2 lists the solubility of Cur-PMEM in different polar and non-polar solvents. It was found that Cur-PMEM showed acceptable solubility profile in PBS as well as organic solvents commonly used in formulations. Table 3 lists solubility of Cur-PMEM polymeric prodrugs in distilled water. Aqueous solubility of curcumin at neutral pH has increased by 45, 53, 60, 75 times compared to native drug in GCU 15, GCU 11, GCU 17, GCU 18 respectively. High proportion of curcumin linking decreases aqueous solubility whereas larger proportion of polymer increases solubility. Depending on the final usage, tailor made chemical conjugation can be done to obtain desired profile of curcumin polymeric prodrug of Cur-PMEM.

**Table 2: Solubility profile of Cur-PMEM and Curcumin in various solvents.**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Curcumin</th>
<th>Cur-PMEM conjugates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>Soluble</td>
<td>Soluble</td>
</tr>
<tr>
<td>DMSO</td>
<td>Soluble</td>
<td>Soluble</td>
</tr>
<tr>
<td>Hexane</td>
<td>Not soluble</td>
<td>Not soluble</td>
</tr>
<tr>
<td>PBS pH 7.4</td>
<td>Soluble</td>
<td>Slightly soluble</td>
</tr>
<tr>
<td>0.1M NaNO₃</td>
<td>Not soluble</td>
<td>Soluble</td>
</tr>
<tr>
<td>THF</td>
<td>Soluble</td>
<td>Slightly soluble</td>
</tr>
<tr>
<td>Water</td>
<td>Not soluble</td>
<td>Soluble</td>
</tr>
</tbody>
</table>
Table 3: Solubility estimate of Curcumin in grafted polymers in distilled water. All values expressed as Mean + SD (n=3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>0.27 ± 0.03 g/L</td>
</tr>
<tr>
<td>Curcumin in GCU 15</td>
<td>12.27 ± 0.5 g/L</td>
</tr>
<tr>
<td>Curcumin in GCU 11</td>
<td>14.54 ± 0.64 g/L</td>
</tr>
<tr>
<td>Curcumin in GCU 17</td>
<td>16.44 ± 1.2 g/L</td>
</tr>
<tr>
<td>Curcumin in GCU 18</td>
<td>20.41 ± 1.8 g/L</td>
</tr>
</tbody>
</table>

Mucoadhesive properties

The force of mucoadhesion defines the mucoadhesive potential of any given polymer. It is obtained by the weight required to detach the polymer from the mucosal surface. The force of mucoadhesion of Cur-PMEM was related to the extent of hydrogen bonding taking place between the polymer and the mucosal surface.

Figure 5: A-Detachment force for mucoadhesion of various Cur-PMEM conjugates and virgin Gantrez AN on excised Sprague Dawley rat small intestinal mucosa determined via double beam balance. All values expressed as Mean + SD (n=3). Statistical analysis performed by unpaired t test comparing decrease in mucoadhesive strength of conjugates with respect to Gantrez AN * P<0.0001, **P<0.001. B-Functional groups (pink) of polymeric conjugate contributing to mucoadhesion via hydrogen bonding.

Cur-PMEM demonstrated good mucoadhesive strength (Figure 5). This could be attributed to the presence of carboxylic group and the methoxy functionality of poly(methylvinyl ether/maleic acid) combined with functionalities capable of hydrogen bond formation within curcumin itself such as the hydroxyl, methoxy oxygen of keto group; in addition oxygen of ester group of the synthesized polymer. Moreover, the high molecular weight average imparted good mucoadhesive characteristics to the graft copolymer. However, a decrease in mucoadhesive property of synthesized conjugates were seen when compared to original
copolymer, this could be attributed to lesser carboxylic acid groups on the grafted conjugates, which are utilized in ester bond. Additionally, hydrophobicity imparted by curcumin molecule could also lead to decrease in mucoadhesion with higher GCU conjugation. Carboxylic acid has greater capacity to form hydrogen bonding with mucin glycoproteins.\[^{28}\] This polymer could have probable applications in mucoretentive, dental, gingival treatments and topical drug delivery systems.

Considering the properties of different curcumin conjugates, GCU11 having highest curcumin percentage with usable mucoadhesive potential was selected for further study.

**Hydrolytic stability studies**

Curcumin undergoes rapid molecular defragmentation under physiologic pH conditions. Degradation of curcumin in aqueous solution has been linked to hydrolysis in alkaline media and experiment was carried out to study hydrolytic degradation of GCU11 and curcumin at pH 7.4 (Figure 6). It was observed that curcumin degradation occurs rapidly at a pH above neutral. The conjugate enhanced the stability of curcumin at pH 7.4. This property could be valuable in dental and oral antimicrobial treatment.

![Figure 6 Hydrolytic stability of GCU 11 at pH 7.4. All values expressed as Mean + SD (n=3).](image)

**Surface morphology and size of nanoparticle**

The surface morphology of nanoparticle prepared with GCU 11 is spherical in shape as seen in Figure 7, size analysed as 350±8 nm, polydispersity index of 0.3 by particle size analyser.
Differential Scanning Calorimetry (DSC)

Figure 8: DSC thermograms of a) Curcumin, b) Poly (methylvinyl ether/maleic anhydride) and c) Curcumin-g-gantrez (Nanoparticle of G-CU11).

DSC thermograms (Figure 8) of Curcumin, poly (methylvinyl ether/maleic anhydride) and nanoparticle of GCU11 showed different patterns. The thermogram of poly (methylvinyl ether/maleic anhydride) showed an endothermic peak around 155°C, which represented the glass transition temperature (Tg), whereas the DSC thermogram of nanoparticle of GCU11 showed an endothermic peak at about 145°C, indicating a shift in the Tg from the original copolymer.

Photodynamic anti-microbial assay

Curcumin has antibacterial and antifungal activity against pathogenic bacteria and fungus, and is therefore prescribed for infectious diseases. Cur-PMEM was therefore expected to inherit the antimicrobial properties of curcumin. To determine the antibacterial action of the conjugate, two bacterial strains: gram negative E. coli and gram positive S. aureus; and for antifungal activity one fungal strain C.albicans that commonly cause infections were selected. (P-L-) and (P-L+) wells did not show any inhibition in microbial growth due to absence of antimicrobial agent. However (P+L+) and (P+L-) wells showed inhibitory action due to presence of curcumin. PMEM did not have any antibacterial activity of its own. However, upon conjugation with curcumin, remarkable antibacterial activity against all the strains (Figure 9) was seen in the resultant polymer at low minimum inhibitory concentration. Photosensitizers exhibit a characteristic absorption spectrum. Upon irradiation of cells containing the photosensitizer with a specific wave length of light corresponding to the absorbance maxima of the sensitizer, the electronic configuration of the sensitizer is raised to a higher energy level (excited state). This excess energy can be converted to heat, to fluorescence emission, or via an intersystem crossing to the "triplet" state from which energy
can be transferred to oxygen in tissues. This results in the formation of "singlet" molecular oxygen, highly reactive oxygen species, such as the superoxide and peroxide anions,\(^{[29]}\) which then attack cellular targets. Although possessing a short lifetime of approximately \(10^{-6}\) seconds, a sufficient concentration of highly cytotoxic singlet oxygen is produced to induce irreversible cell damage. In addition, the photosensitiser is not necessarily destroyed, but can return to its ground state by phosphorescence without chemical alteration and may be able to repeat the process of energy transfer many times. Alternatively, the sensitiser may return to ground by transferring its energy to molecular oxygen, and may even be destroyed by photobleaching due to oxidation. Evidently, many effects of PDT are oxygen dependent and rely on the oxygen tension within the target tissue. Singlet oxygen is, however, widely believed to be the major damaging species in PDT. It can travel approximately 0.1 µm inside the tissue. This means that the damage is localized to the area containing the photosensitiser.

The MIC was remarkably reduced compared to plain curcumin. This augmented activity was seen due to enhanced aqueous solubility of conjugate. The photodynamic potential of GCU11 and curcumin is highlighted from the study. Curcumin is a photosensitizer and gets activated at 460nm. It could be anticipated that \textit{invivo}, nanoparticle of GCU11 would provide better bioavailability and therapeutic effect than curcumin alone due to improved aqueous solubility and stability, with great reduction in therapeutic dose. Use of LED, non-laser source has been commonly used and reported for the irradiation of easily accessible skin, oral and dentine surfaces.\(^{[27]}\) This technique is cheap, flexible, lightweight, can be smartly arranged as per the experimental design without use of sophisticated instruments.\(^{[30]}\)

Figure 9: Photodynamic antimicrobial activity of Gantrez AN, Curcumin and Nanoparticle of GCU11 against \textit{S.aureus}, \textit{E.coli} and \textit{C.albicans} in presence(L+) and absence(L-) of LED. All the experiments were performed in triplicates for each group. Gantrez AN showed no antimicrobial activity against microbes. P-L- and P-L+ did not show any activity, where P is the photosensitizer. Statistical analysis by Nonparametric post hoc test (*P < 0.05, **P<0.001).
CONCLUSION
We report here successful design and development of curcumin-graft-poly(methylvinyl ether/maleic acid conjugate by esterification. GCU 11 was selected as promising candidate with good aqueous solubility and hydrolytic stability. The nanoparticle prepared with this amorphous conjugate has exhibited phenomenal photodynamic therapy against microbes. The esterified curcumin conjugate has demonstrated effective prodrug strategy for antimicrobial purposes with mucoadhesive property and can be tailor made for topical, dental or oral applications for immediate or sustained delivery. The final product can be scaled up and is economical.

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