Formulation and Evaluation of Biodegradable Sustained Release Aceprophyline Cow Urine Nanoparticle for the Treatment of Asthma

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ABSTRACT
The objective of present investigation was to evaluate the entrapment efficiency of the anti-asthma drug Aceprophyline, using natural polymer of different ratio (1:1 to 1:3) and study the effect of this entrapment on the drug release properties. The present study relates to such precious and holy animal derived material cow urine used as a medium and improves the anti-asthmatic activities. The morphology of the nanoparticles was evaluated using a scanning electron microscope, which showed a round and spherical shape with smooth surface. The result of ratio (1:3) showed a good encapsulation efficiency of 98.29%. Aceprophyline nanoparticles was confirmed by FTIR, DSC and quantitated by UV prepared nanoparticles appeared spherical with round drug core in transmission electron microscopy studies. The release rate of Aceprophyline from sodium alginate cow urine nanoparticles was much lower than that HPMC K100 cow urine nanoparticles. Evaluation of release data reveals that release of Aceprophyline with cow urine nanoparticles followed the zero orders, whereas sodium alginate cow urine nanoparticles shows as sustained dispersive drug release was observed invitro one releasing the drug pay load over a period of 16hr. embedding Aceprophyline nanoparticles in alginate provided sustain release. They also offered better pharmacokinetic properties to the drug than afforded by the free drug itself. The cow urine nanoparticle method developed a good choice for one development of sustained anti-asthmatic drug therapy. Improve the patient compliance, reduce the side effects.

KEYWORDS: Cow urine nanoparticles, Aceprophyline, sodium alginate, HPMC K100, solvent evaporation, cross linking method.

INTRODUCTION
Gir cow are very well known high midst of India. The breed is also recognized as having high resistance towards many infections. The cow urine plays an important role in diagnosis of many diseases. From the ancient period cow’s urine used as a medicine Cow urine this kind of alternative treatment, termed as reported to be beneficial even for dreaded diseases like Cancer, Aids, and Diabetes. [1] It is an airway mucus regulator with anti-inflammatory action. The drug’s approach involves several points of attack in obstructive airway disease. The molecule contains ambroxol, which
facilitates various steps in the biosynthesis of Acebrophylline of pulmonary surfactant, theophylline-7 acetic acid whose carrier function raises blood levels of ambroxol, thus rapidly and intensely stimulating surfactant production. The resulting reduction in the viscosity and adhesivity of the mucus greatly improves ciliary clearance. By deviating phosphatidylcholine towards surfactant synthesis, making it no longer available for the synthesis of inflammatory mediators such as the leukotrienes, acebrophylline also exerts an inflammatory effect. This is confirmed in vivo by the reduction in aspecific bronchial hyper-responsiveness in patients with stable bronchial asthma. On a clinical level, aceprophylline is therapeutically effective in patients with acute or chronic bronchitis, chronic obstructive or asthma-like bronchitis and recurrence of chronic bronchitis; it reduces the frequency of episodes of bronchial obstruction and reduces the need for beta2-agonists, and improves indexes of ventilatory function.

One of the popular methods for the entrapment of drugs within water soluble polymer is the chemical cross-linking method. Therefore, the purpose of present study is to incorporate the anti-HIV drugs, aceprophylline cow urine nanoparticles using two polymers of different concentration as well as permeability characteristic at different polymer solution concentration, in order to evaluate the entrapment efficiency and nanoparticle properties especially their dissolution release characteristics.

EXPERIMENTAL MATERIALS
Aceprophyline was obtained as a gift sample from Caplin point Pondicherry (India). Sodium alginate (250cps), HPMCK100 was purchased from sigma aldrich, Bangalore. All other reagents of analytical grade were purchased from merck or spectrochem and used.

METHOD
NANO EMULSIFICATION POLYMER-CROSS LINKING METHOD
10mg of drug, Aceprophyline was taken in 10ml of methanol and emulsified under sonication at 20 kHz in 30ml of 0.1% m/v aqueous solution of sodium alginate, using tween80 as emulsifier. Glycerol used as stabilizer (~5ml) was added in the reaction mixture to effect cross linking of the nanoparticle produced. The reaction mixture was cooled for 24hrs at room temperature (25°C) nanoparticle were then separated by ultra-centrifugation at 20,000rpm, 0c,30min, mps thus obtained were washed with 15ml of water, recentrifuged these were then preserved in vacuum decicator at 4°C for further evaporation.

SOLVENT EVAPORATION METHOD
Aceprophyline was dissolved in methanol and polymer solution was emulsified into 25ml of 2% polyvinyl alcohol, using a probe Sonicator set at 55W energy output for 2 mins over an ice-bath to form an oil/water emulsion. The resulting emulsion was kept stirring overnight at 4°C to evaporate the methanol. Nanoparticles were recovered by ultracentrifugation at 30,000 rpm for 20 min at 4°C and washed thrice with distilled water to remove polyvinyl alcohol and encapsulated Aceprophyline. The pellet was resuspended in water, sonicated for 30 sec, and centrifuged at 1000 rpm for 10 min at 4°C. The supernatant was collected, frozen at -70°C and lyophilized for 48 h to form a dry powder.

LOADING EFFICIENCY
For determination of loading efficiency the amount of drug present in the clear supernatant after centrifugation was determined by UV-Visible spectro photometry. A standard calibration curve of concentration versus absorbents was plotted for this purpose. The amount of drug in the supernatant was than subtracted from the total amount of drug loaded during co-acervation process. The presence of loading efficiency of nanoparticle was calculated by the formula (w-w1) x100/w.

ZETAPOTENTIAL
Zetapotential was measured for each formulation using large bore capillary cells in the zeta sizer nano-2s (Malvern instruments); 1ml of nanoparticle suspension from the proportion medium was sampled out and diluted to 5ml with (0.9%) m/v sodium chloride solution prepared in distilled water for optimal signal intensity. Three formulations were recorded to get the average zetapotential for different formulation.
Measurement Results

201410111245011.nzt
Measurement Results
Date: Saturday, October 11, 2014 12:45:14 PM
Measurement Type: Zeta Potential
Sample Name: F1
Temperature of the holder: 25.1 °C
Viscosity of the dispersion medium: 0.893 mPa·s
Conductivity: 2.091 mS/cm
Electrode Voltage: 2.8 V

Calculation Results

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Zeta Potential</th>
<th>Electrophoretic Mobility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-18.3 mV</td>
<td>-0.000128 cm²/s</td>
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<tr>
<td>2</td>
<td>-16.5 mV</td>
<td>-0.000128 cm²/s</td>
</tr>
<tr>
<td>3</td>
<td>-15.3 mV</td>
<td>-0.000128 cm²/s</td>
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</table>

Zeta Potential (Mean): -16.5 mV
Electrophoretic Mobility mean: -0.000128 cm²/s
Measurement Results

201410111249012.nzt

Measurement Results
Date: Saturday, October 11, 2014 12:49:19 PM
Measurement Type: Zeta Potential
Sample Name: F2
Temperature of the holder: 25.0 °C
Viscosity of the dispersion medium: 0.896 mPa.s
Conductivity: 2.178 mS/cm
Electrode Voltage: 2.8 V

Calculation Results

<table>
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<th>Peak No.</th>
<th>Zeta Potential</th>
<th>Electrophoretic Mobility</th>
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<tbody>
<tr>
<td>1</td>
<td>-9.7 mV</td>
<td>-0.000075 cm²/s</td>
</tr>
<tr>
<td>2</td>
<td>-mV</td>
<td>-cm²/s</td>
</tr>
<tr>
<td>3</td>
<td>-mV</td>
<td>-cm²/s</td>
</tr>
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</table>

Zeta Potential (Mean): -9.7 mV
Electrophoretic Mobility mean: -0.000075 cm²/s
Measurement Results

201410111329021.nzt
Measurement Results

Date: Saturday, October 11, 2014 1:29:42 PM
Measurement Type: Zeta Potential
Sample Name: F3
Temperature of the holder: 25.0 °C
Viscosity of the dispersion medium: 0.894 mPa.s
Conductivity: 2.081 mS/cm
Electrode Voltage: 2.8 V

Calculation Results

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Zeta Potential</th>
<th>Electrophoretic Mobility</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>-2.3 mV</td>
<td>-0.000018 cm²/s</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Zeta Potential (Mean): -2.3 mV
Electrophoretic Mobility mean: -0.000018 cm²/s
IN-VITRO DRUG RELEASE STUDIES

In-vitro drug release studies were carried out for all products using dialysis tubes with an artificial membrane. About 10mg of accurately weighed NPS were taken in 5ml of pH 6.8 phosphate buffer was added to the dialysis tube. The release studies were carried out at 37°C under continuous stirring at 120rpm and cumulative drug was measured under the sink condition. 5ml aliquots were sampled out a regular time interval was measured at UV-spectrophotometrically280nm. The data obtained were fitted into various kinetic models to investigate the mechanism of drug release from cow urine nanoparticles.

RESULTS AND DISCUSSION

DSC ANALYSIS

DSC is very useful in the investigation of the thermal properties of drug delivery concern, providing both qualitative and quantitative information about the physico chemical state of inside the drug delivery system. In the present study, DSC thermo grooms for Aceprophyline, Aceprophyline and sodium alginate, Aceprophyline and HPMCK100 and nanoparticles. As shown in Fig 1 to 3 melting endotherm of pure Aceprophyline and Aceprophyline and sodium alginate with drug polymer mixture showed the presence of endothermic peak at 153.6, 172.99 respectfully in addition sharp endothermic peak were observed at Aceprophyline and sodium alginate polymer 173.4°C. DSC thermo grooms showed that there was no mixer differences in onset Temp and end set Temp and compared with pure thermo groom peaks. No interaction was found between drug polymer peak. No interaction was found between drug and polymer.
IR –SPECTRUMS OF ACEPROPHYLINE NANOPARTICLES

For the I.R spectrum of Aceprophyline characteristic peak at 3449cm⁻¹ un times the presence of NH stretch, primary amine C=O stretching respectively in the I.R spectrum of Aceprophyline the primary amine 3118cm⁻¹ strong NH stretching at 2547,1547cm⁻¹ were observed. For I.R spectrum of Aceprophyline nanoparticles it is observed that vibration of the NH and OH bending at 3289 and 3210cm⁻¹ was present. From the I.R spectrum of the nanoparticles on comparing with spectra of Aceprophyline some peaks become stronger due to interaction of peak amino groups of Aceprophyline 3416 and NH bending vibration C=C stretching 1476scm⁻¹ were observed respectively. No drug – polymer chemical interaction in loaded nanoparticles.

Fig. FTIR graph for pure Aceprophyline
FORMULATION OF NANOPARTICLE BY VARIOUS METHOD

Aceprophyline loaded alginate NPS were prepared following nano suspension and six polymers cross linking. The three different formulations were designed (table-I) using difference drug polymer ratio such as (1:1-1:3) different drug polymer ratio have shown a distant impact on the drug pay loading and nanoparticles size distribution. Both the size and size distribution can strongly influence nanoparticular drug delivery.
### Table-I: Formulation design and drug payload for aceprophylline cow urine nanoparticles

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Solvent/oil phase</th>
<th>Drug/aq.sol chitosan</th>
<th>Tween80</th>
<th>CaCl2 sol.</th>
<th>PVA</th>
<th>Drug load (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Methanol</td>
<td>1:30.0</td>
<td>0.09</td>
<td>2.5</td>
<td>--</td>
<td>98.29±0.00</td>
</tr>
<tr>
<td>B</td>
<td>Methanol</td>
<td>1:20.0</td>
<td>0.09</td>
<td>2.5</td>
<td>--</td>
<td>94.69±0.24</td>
</tr>
<tr>
<td>C</td>
<td>Methanol</td>
<td>1:10.0</td>
<td>0.09</td>
<td>2.5</td>
<td>--</td>
<td>90.35±0.52</td>
</tr>
<tr>
<td>D</td>
<td>Methanol</td>
<td>1:30.0</td>
<td>--</td>
<td>--</td>
<td>10</td>
<td>80.34±0.48</td>
</tr>
<tr>
<td>E</td>
<td>Methanol</td>
<td>1:20.0</td>
<td>--</td>
<td>--</td>
<td>10</td>
<td>78.14±0.75</td>
</tr>
<tr>
<td>F</td>
<td>Methanol</td>
<td>1:10.0</td>
<td>--</td>
<td>--</td>
<td>10</td>
<td>75.25±0.58</td>
</tr>
</tbody>
</table>

**Mean ±SE, n=6**

Size distributions for all formulation were studied in pcs and the mean pcs diameters (table-II) were directly recorded as intensity weighted. Pcs size distribution of formulation A provided Gaussian size distribution with an average nanoparticle diameter 270nm (fig.1). Size distribution B was partially skewed average diameter was 276nm. Formulation C produced larger particles of average pcs 171nm respectively.

### Table-II: Studies of different formulations in PCS

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Particle size(nm)</th>
<th>Poly dispersityindex</th>
<th>Zeta(mev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>270.5</td>
<td>3.0±0.00</td>
<td>-16.0±0.00</td>
</tr>
<tr>
<td>B</td>
<td>276.2</td>
<td>21.0±0.12</td>
<td>9.7.0±0.2</td>
</tr>
<tr>
<td>C</td>
<td>171.8</td>
<td>2.13±0.012</td>
<td>2.3±0.0</td>
</tr>
</tbody>
</table>

**Mean ±SE; n^a=4, n^b=6**

Polydispersely index (PI) was recorded as an index for particle size distribution in prepared formulations. PIs ranged from monodispersed 0.000 to 1.000, where PI is greater than 0.500 indicated relatively broader distribution PIs near 0.250 are generally considered ideal. PIs of formulation A (16.0±0.00), formulation B (9.7±0.2) were most suitable for circulating NPS.

Particle size determination of Formulation A
Particle size determination of Formulation B

Particle size determination of Formulation C

ZETAPOTENTIAL

Zeta potential is an important physiochemical parameter which can influence the stability of nano-drug carrier formulations. Extremely positive or negative zeta potential values cause large repulsive force, while electrostatic repulsion between particles with the same electric charge prevents aggregation of the sphere. Negative zeta potential values ranging from -3.0 to -0.9 mev were observed in three formulations.

Table III: Kinetic evaluation of drug release data for nanoparticle formulation

<table>
<thead>
<tr>
<th>Release kinetic model</th>
<th>Zero order</th>
<th>Higuchi model</th>
<th>Power law</th>
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</thead>
<tbody>
<tr>
<td>Equation K value</td>
<td>Dt=kt</td>
<td>Q=kQt^{1/2}</td>
<td>Mt=mgk_pn^4</td>
</tr>
<tr>
<td></td>
<td>K_t=0.0236</td>
<td>K_q=(6.9±0.4)x10^{-5}</td>
<td>K_p=0.2429±0.0044</td>
</tr>
<tr>
<td></td>
<td>R^2 = 0.9665</td>
<td>0.9045</td>
<td>n=0.4315</td>
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</table>

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TEM is a 2D image of a 3-dimensional nanoparticle while PCS provided NPS hydrodynamic diameter in terms of equivalent sphere. Transmission electron microscope of urenyl acetate stained formulation. A NPS were spherical with a dense core of encapsulated Aceprophyline

Average TEM diameter of formulation A was observed to be 200nm counts in two observation plates. TEM diameter through appeared relatively larger they were with in higher ranges of PCS observation. Both PCS and TEM are independent techniques of observation, but were sufficiently informative and complementary to each other. A cumulative percentage drug release profile of formulation A was studied (fig.3).

A sustained release profile for Aceprophyline payload was observed over a period of 16hr. Both the size and the amount of the drug loading where known to influence the nanoparticular drug release profile. In
general, the drug release mechanism of formulation A drug release rate did not, however, fit into the concentration and time dependent first order release mechanism model, suggesting that the overall in-vitro Aceprophyline release kinetic mechanism was of payload zero order type, as expected for particular water soluble drug carrier. As the drug loading in formulation a 98.29% the surface area dependent on the ‘n’ value when ‘n’ value is 0.43, drug diffusion is professional to the concentration gradient. Indicating fickian diffusion while ‘n’ value reaching 0.85 is indicating of the drug concentration gradient, independent, non-fickian drug release mechanism.

CONCLUSION
Anti-asthmatic nano carrier in biodegradable polymer material, following a sustained release profile, here been formulated significant Aceprophyline payload in alginate NPS was achieved using the drug polymer ratio (1:3) as good loading efficiency. This study confirms that the chemical cross linking method is suitable for the preparation of Aceprophyline nanoparticles with high encapsulation efficiency. This formulation approach can be used to improve the therapeutic efficacy, reduce the side effects, and improve the bioavailability.

REFERENCES

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