6. Discussion of Results

The above research work relates to the preparation of nanoparticles and the evaluation. The drugs used for the study were Amoxicillin, Ampicillin, Ciprofloxacin and the polymers used were sepia (cuttlefish ink) and chitosan. The above said drugs possess a less biological halflife which demands for the frequent administration leading to dose dumping finally. So in this work, an oral controlled release formulation was made through the preparation of nanoparticles. The nanoparticles which are smaller in size have also been known to be a very good carrier of the drug molecules.

The carrier used in the above formulation was obtained from a marine source. The sepia is non-toxic containing melanin pigments. The unique characteristics of the sepia include its biocompatibility and good binding capacity with Aminoglycosides and Fluroquinones. The sepia finds application as a food colouring agent and in the preparation of pastries and sauces.

The drug and polymer ratios formulated were 1:1, 1:15, 1:2, 1:2.5, 1:3, 1:3.5 and 1:4.

6.1 Physicochemical properties of polymer sepia

The crude ink obtained from the ink sac was first boiled with caustic soda. To the filtered extract 0.1N HCl was added for precipitating the colouring matter. The liquid of cuttlefish ink has a grainy texture.
The main constituents of the ink are melanin and mucus. The different physicochemical properties of sepia determined are given below:

**Values of PhysicoChemical properties of Sepia**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size</td>
<td>4.475μm</td>
</tr>
<tr>
<td>Bulk density</td>
<td>1.35g/cc</td>
</tr>
<tr>
<td>True density</td>
<td>1.5652g/cm³</td>
</tr>
<tr>
<td>Porosity</td>
<td>14%</td>
</tr>
<tr>
<td>Angle of Repose</td>
<td>9.211°</td>
</tr>
<tr>
<td>Viscosity</td>
<td>0.606cps</td>
</tr>
<tr>
<td>Surface tension</td>
<td>13.2521dynos/cm²</td>
</tr>
<tr>
<td>Melting point</td>
<td>195°C</td>
</tr>
<tr>
<td>pH</td>
<td>6.76</td>
</tr>
</tbody>
</table>

**6.2 Preliminary Compatibility Studies of drugs and polymer**

The drug and the polymer interactions were studied using FTIR. In the IR graph of Amoxicillin, the peaks were obtained in the wavelength regions of 3705.71.

**6.2.1 Ciprofloxacin**

The peak obtained at 1381 cm indicates CF stretching, as the presence of fluorine atom was observed in the range of 1000-1400cm. The hydrogen-bonded acids i.e. COOH group was noticed in the range of 2500-3000 and its peak was obtained at 2809cm. The –C- ketone group was present at a range of 3300-3600cm and its peak was at 3412cm.
Moreover, the presence of aminogroup i.e. NH-stretching was confirmed at the range of 3300-3500 and its peak was observed at 3412.49 cm$^{-1}$. CH stretching was observed at the range of 3000-3100 cm$^{-1}$ which indicates the presence of aromatic rings and the alkenes at the peak of 3082.62 cm$^{-1}$.

The presence of alkene group was observed at the peak of 669 cm$^{-1}$. The OH group was confirmed at a peak of 3000.79 cm$^{-1}$. The nitrile group i.e. C=N which is an aromatic ring was found at a peak of 2228 cm$^{-1}$. Interestingly, the above functional groups of Ciprofloxacin and the excipients showed more or less same peaks at wavelengths such as 1381.20, 2816.41, 3408.63 and 3802.62. By comparing the IR spectra of the pure drug and its physical mixture, it may be concluded that there was no interaction between the drug and the excipients.

**6.2.2 Amoxicillin**

In the case of the pure drug Amoxicillin, a peak was noticed in the wavelength region of 3032.47 which is however, due to the presence of C-H stretching, Similarly, aromatic rings were obtained in the regions of 2816.41 (due to C=O stretching), 3414.42 (due to N-H stretching), 1336.83 (due to C-N stretching) and 3464.57 (due to the phenols-OH stretching). For the physical mixture of drugs, the peaks were obtained in the wavelength regions of 3262.95, 2820.27, 1680.26, 3497.37, 1343.69 and 3595.28. Interestingly, the mixture of the pure drug and the excipients showed more or less similar peak as that of the pure drug. It
is therefore confirmed that the drug Amoxicillin is compatible with excipients.

**6.2.3 Ampicillin**

In the Ampicillin-loaded formulations, the peaks were obtained at wavelength regions such as 2968 (due to the C-H stretching), 3398 (due to the N-H bond), 1689.85 (due to C=O), 1251.67 (due to C-C), 1373.48 (due to -C-N stretching) and 3275.53 (due to C-H stretching aromatic). Similar peaks were also obtained for the physical mixture of the drug at wavelength regions such as 2968.81, 3441.43, 1691.70, 1263.93, 1375.41 and 3210. Interestingly, the mixture of the pure drug and the excipents exhibited more or less similar peaks as pure drugs. Hence, it could be confirmed that this drug is compatible with the excipients.

**6.2.4 Ofloxacin**

In this drug formulation, the peak which was obtained at 1932.91 cm indicates the presence of a ketone group. The C-F stretching was mainly due to the presence of fluorine atom attached a benzene ring which was noticed in association with a peak at a wavelength region of 952.95. Moreover, the C-H stretching i.e. C-H bond, aromatic ring and the presence of alkene could be confirmed at a peak obtained at the wavelength region of 3022.04. Peaks noticed at 3341.12 wavelength region confirmed the presence of OH group. The C=N was noticed with the peak obtained at the wavelength region of 1550.96 and the peak at the wavelength region of 3412.49 was largely due to the presence of an
aminogroup i.e. NH stretching. Similar peaks were obtained in the case of physical mixture of drugs too. It is again confirmed that there was no interaction between the drugs, Ofloxacin with other excipients.

6.3 *in-vitro drug release characteristics*

The different nanoparticle formulations were prepared using various drugs and polymers at concentrations such as 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4 and 28. The prepared formulations were subjected to dissolution studies. The percentage of drug release with respect to the time was recorded in all these formulations.

In the case of Amoxicillin nanoparticles, upon increasing the concentration of the polymer, the formulations generally showed good release pattern however, up to the concentration of 1:2. The release rate was gradually decreasing in other higher concentrations. Hence, the AMN3 is considered to be an ideal formulation, as it showed good release profiles. i.e. in 15min, 47% of drugs was found to be released and at 24th hour, the drug release was 90.36%.

In the Ciprofloxacain nanoparticles, the formulation CN3 was found to be the best as at the 24th hour, the drug release was 85%. The drug release profiles of CN3 formulation showed that it was 39.25% in 15min and 50% in 4hrs.

In the Ampicillin nanoparticles, upon increasing the concentration of the polymer, the increased drug release was observed in the drug and polymer concentration of 1:3. On the other hand, the release rate was
found slowly decreasing at a drug-polymer concentration of 1:4. Further, AP5 is considered to be an ideal formulation as 88% drug release was observed in this formulation at the 24th hour.

The drug release studies of the Ofloxacin nanoparticles further showed that the OF6 could be an ideal formulation as there was 87% drug release at the 24th hour.

**6.4 Size analysis of nanoparticles**

The formulations which showed good release profiles were subjected to size analysis using Scanning Electron Microscope (SEM). The size of the nanoparticles of Ciprofloxacin and Amoxicillin was found to be 500nm and that of Ofloxacin and Ampicillin was found to be 100nm. It is also worthy of mention here that the sizes (100 and 500nm) of nanoparticles prepared and used for the present research work were within the size range (1-1000nm) of nanoparticles prepared and experimented in earlier works.

**6.5 Drug entrapment studies**

In the prepared Amoxicillin nanoparticles, more than 50% of drug was found to be entrapped in the carrier matrix. The formulation AMN3 showed a good release pattern as 65.33% of drug got entrapped. In the case of prepared formulation of Ciprofloxacin nanoparticles, more than 55% of drug was getting entrapped and in the CN3 formulation, 67% of drug was found to be entrapped. In the Ampicillin nanoparticle formulation, above 52% drug got entrapped and the AP5 formulation
with the good release profile showed 62.13% of drug entrapment. In the Ofloxacin formulation, OF6 which showed good release pattern, 63.12% of drug was found to be entrapped.

6.6 Zeta potential analysis

The formulaions such as AMN3, CN3, AP5 and OF6 were subjected to zeta potential analysis in order to find their surface charge and the obtained values in this regard were found to be 69mv, 58mv, 52mv, 63mv respectively.

6.7 Stability studies on selected formulations

The stability studies were performed for the selected formulations such as AMN3, CN3, AP5 and OF6 as per ICH guidelines. These formulations were packed in screw capped bottles and were stored at different temperatures viz. room temperature, 45°C and 4°C for one month and the samples were analysed at weekly intervals.

In the case of Amoxicillin nanoparticles, the degradation was found to start while storing at room temperature and the efficiency of the drug was at 97.43% in this temperature. On the other hand, 94.75% of drug could be observed in the case of Amoxicillin nanoparticles (AMN3) stored at 4°C. Further, at the end of 4 weeks, the values of concentration of drugs stored at room temperature, 45°C and 4°C were found to be 93.65%, 88.86% and 92.65% respectively. It is therefore concluded that the room temperature is ideal for storing AMN3 formulation.
In the Ciprofloxacin nanoparticles, there was no change in the drug concentration when they were stored at the room temperature and 4°C. However, when the sample was stored at 45°C, the concentration of drug was found to reduce considerably (90.72%). At the 4th week, the samples stored at room temperature and 4°C, the values of concentration of the drug, Ciprofloxacin were 94.38% and 98.20% respectively. It was also noticed that the drug concentration was 88.93% when the sample was stored at 88.93%.

The accelerated stability studies were also performed for the Ampicillin nanoparticles (AP5) and the Ofloxacin nanoparticles (OF6) and it was found that there was no change in the concentration of these drugs at room temperature and at 4°C during the first week of storage. But at a storage temperature 45°C, the degradation was found to start and the values of concentration of the drugs during the first week were 99.50% and 98.50% for AP5 and OF6 respectively. The values of concentration of the drug in the formulation AP5 at the 4th week in room temperature, 4°C and 45°C were 96.1%, 97.7% and 94.6% respectively. Further, all the formulation of AMN3, CN3, AP5 and OF6 were found to be stable on prolonged storage.

6.7 Differential scanning calorimeter analysis

Through the Ciprofloxacin DSC graph, one could see the values of onset peak temperature, endset temperature and the peak temperature as 173.4°C, 208.72°C and 192.37°C respectively. Similar peaks were also
obtained in the case of the prepared formulations of Ciprofloxacin. In the DSC graph of the Amoxicillin, the values of onset peak temperature, endset temperature and peak temperature were 114.15°C, 147.72°C and 137.14°C respectively. But in the case of Amoxicillin formulation, the onset temperature was 144.32°C, the endset temperature was 182.27°C and the peak was obtained at 168.14°C. For the Ampicillin formulation, the onset of peak was at 100°C. The values of endset temperature and peak temperature were 140°C and 150°C respectively. Similar values were more or less observed in the Ampicillin formulation also. For the Ofloxacin formulation, the onset peak was at 250°C and endset peak was at 300°C. On the otherhand, a sharp was obtained at 275°C and the peak was at 280°C. By comparing the DSC graph of the drugs and the respective formulations, it is understood that there was no or nil interaction between the drug and the carrier.

6.8 Pharmacokinetic evaluations

In-vivo evaluation was performed for the selected formulations namely viz. AMN3, CN3, AP5 and OF6.

The drug release pattern of Amoxicillin formulation, AMN3 was 1.78µg/ml after 15min of oral administration. The peak plasma concentration in this case was 2.75µg/ml at the 24th hr and after that, the elimination started and the concentration of drug release at 72nd hr was 1.92µg/ml. The drug release in all these cases was in a sustained manner. For AMN3 formulation, the values of absorption constant,
elimination constant (ke) and half-life were 0.0069, 0.634 and 10.43 hour respectively. On the other hand, the values of the Tmax, apparent volume at distribution, Cmax and AUC in respect of AMN3 formulation were 39.23 hr, 3.76 l, 8.6 µg/ml and 130.58 respectively.

For the formulation CN3, the mean concentration of drug in blood plasma after 15 min of administration was 1.5 µg/ml and the peak plasma concentration obtained at 24th hr was 3.19 µg/ml. After that, the elimination was found to start. A plasma drug concentration of 0.775 µg/ml at the 72nd hr showed that the drug release from the nanoparticle was in a sustained manner. For this formulation, the values of half-life, absorption constant (ka), elimination constant and Tmax were found to be 17.325, -0.04, 0.0732, and 36.6 hr respectively. The values of apparent volume of distribution, Cmax and AUC were at 3.62 hrs, 31.5 µg/ml and 209.7 µg/ml respectively in this formulation.

In the case formulation OF6, the concentration of drug in blood plasma after 15 min of oral administration was 1.76 µg/ml and the peak plasma concentration was 3.26 µg/ml. After that, the elimination was found to start. The amount of drug present at 72nd hr was 1.91 µg/ml. A maximum amount of drug was eliminated at the end of the experimental period. This formulation was also found to release the drug in a sustained manner. The values of elimination rate constant, absorption rate constant, Cmax, Tmax, AUC, and biological half life for
this formulation were 0.0057, 0.0098, 93, 32hrs, 17.8060 and 28.87hrs respectively.

For the formulation AP5, an amount of 1.75µg/ml was found to be released when experimentation was done in the serum blood samples. A maximum drug release was at a concentration of 3.18µg/ml at the 24th hr. After that, the elimination started and at the 72nd hr, and the plasma drug concentration was 0.82µg/ml. The pharmacokinetic parameters reevaluated showed that the values of AUC, ka, ke, t_{1/2} and Cmax were at 18.7013, 0.0133 per hr, 0.0034 per hr, 15.13hrs and 95 respectively.

From the In-vitro data obtained, it is clear that the drug release was in sustained manner in a period of time from 15min to 72hrs. Hence, the above formulations experimented are considered as ideal formulations.