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PREPARATION AND CHARACTERIZATION OF POLY(2-HYDROXYETHYL METHACRYLATE-CO-METHYL METHACRYLATE) HYDROGELS FOR SUSTAINED DELIVERY OF ANTITUMOR DRUG

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INTRODUCTION

Cancer is one of the diseases that contribute to the high mortality rate globally. Thus there has been need for continuous research to develop drugs and formulations to curb this disease. Cancer is fundamentally a disease at the cellular level, in which cell proliferates indefinitely. Consequently, cancer cells continue to grow and divide yielding an ever increasing mass referred to as a tumor. The tumor grows invasively, destroying surrounding body tissues. Cancer cells from this primary tumor may then spread, or metastasize, to other parts of the body, where new tumors may begin to grow. Eventually the tumor load will cause death, often by physically blocking or compressing blood vessels or organs such as the brain.

In the recent years, considerable attention has been focused on the development of Novel Drug Delivery Systems (NDDS). The reason for this paradigm shift is due to the low development cost and time required for developing a NDDS for the existing drugs rather developing a new drug molecule. In the form of NDDS, existing drug molecule can get a new life, there by increasing the market value and product patent life. Implantable drug delivery system (IDDS) is an example of such systems available for therapeutic use.

Cisplatin (*cis*-diamminedichloroplatinum(II), (CDDP) is a commonly used chemotherapeutic agent for treatment of various cancers, including testicular cancer, ovarian cancer, lymphoma, and glioma. After both passive and active cellular uptake, cisplatin coordinates with the N7 atom of guanine in DNA to form adducts and causes cellular apoptosis.

In the present work, Poly(2-hydroxyethyl methacrylate-co-methylmethacrylate) hydrogel based implants were prepared using cisplatin as a model drug, and the characteristics of these implants were investigated. In addition, the effects of concentration of each ingredient in implants and *in vitro* and *in vivo* release behaviour of cisplatin from these films were examined along with the *in vitro* and *in vivo* biodegradation of implants and histopathological studies. The films were studied for thermal characteristics by DSC, for intermolecular interactions by FT-IR and for morphological characteristics by SEM.

MATERIALS AND METHODS

Materials

Cisplatin was procured from Arora Mathew Ltd. HEMA, MMA, EGDMA, Eosins stain solution, Pottasium Dihydrogen orthophosphate, Sodium hydroxide were of analytical grade.

METHODS

Formulation of CDDP implants[1,18,28]

The hydrogel based implants were prepared by using 2-hydroxyethyl methacrylate (HEMA) and Methyl methacrylate (MMA) monomers. Ammonium persulfate and Sodium metabisulphite were used as initiators for polymerization reaction. Ethyleneglycol dimethacrylate was used as cross linking agent. Polymerization was carried out at room temperature and disks of desired size were cut using cork borer. The drug was incorporated before the addition of initiators.

The objective of varying the concentration of cross-linking agents was to achieve an optimized formulation, which would give a sustained release of dug over a period of four weeks.

Table 1: Formulation chart of Cisplatin implants.

Ingredients	F-I	F-II	F-III	F-IV	F-V
Cisplatin (mg)	10	10	10	10	10
2-hydroxyethyl methacrylate(%w/v)	85	80	75	85	85
Methyl methacrylate (%w/v)	15	20	25	15	15
Ethyleneglycol dimethacrylate (%w/v)	2	2	2	1	4
Ammonium persulphate(mg)	150	150	150	150	150
Sodium metabisulphite (mg)	100	100	100	100	100
Water	qs	qs	qs	qs	qs

Preparation of implants[1,18,28]

Step I: Both the monomers HEMA and MMA were added to 10 ml of water with stirring for 2 min.

Step II: To the above solution an aqueous solution of ammonium persulphate (APS) and sodium metabisulphate (SMB) were added and allow stirring for 1 min.

***Step III*:** A known concentration of EGDMA solution was added as a cross linking agent with stirring.

Step IV: The resultant solution was cast on Petri dish, and allowed to dry at 37^{o}C for 3 days.

Step V: Prepared implants were taken for further studies.

Characterization: The prepared implants were characterized by Fourier transform infra red spectroscopic analysis, Differential scanning calorimetric analysis and Scanning electron microscopic studies.

The FT-IR spectral measurements were taken at ambient temperature using Shimadzu FTIR, Japan. About 2 mg of the pure drug and optimized formulations were selected separately. Pure drug and formulations were dispersed in KBr powder and the pellets were made by applying 6000 Kg/cm^2 pressure. FT-IR spectra were obtained by powder diffuse reflectance on FT-IR spectrophotometer.[2,14]

DSC[19] measurements were performed on a DSC Dupont 9900, differential scanning calorimeter with a thermal analyzer. Samples with weights between 2 and 5 mg were then sealed into an aluminium pan. An empty aluminium sealed pan was used as reference material. The temperature was raised from 10 to 400,C at a heating rate of 10,C/min under nitrogen atmosphere (flow 10 ml/min).

SEM was done using Joel SEM analysis instrument Japan. It was done to study external morphology of implants before and after swelling; samples were mounted on aluminum mount, using double-sided adhesive tape and sputtered by gold under vacuum and were scanned at an accelerating voltage of 15KV before observation.

Evaluation

Swelling studies[1,18,21,30]

The swelling characteristics of copolymeric poly (HEMA: MAA) hydrogel based implants synthesized using various concentrations of EGDMA were determined by immersing weighed test samples in 25 ml phosphate buffer solution of pH 7.4 at 37^{0}C separately. At specific time intervals, samples were removed from the swelling medium and blotted with a piece of paper for 5 seconds to absorb excess water on surface and they reweighed. Studies were carried out in triplicate.

The swelling ratios (*Qs*) of the test samples were calculated from the following Equation.

$Qs = (Ws - Wd) / Wd$

Where *Ws* is the weight of the swollen test sample and *Wd* is the weight of the dried test sample.

Diffusion Coefficient

The diffusion coefficient was calculated to measure the penetration rate of water molecules into the implant and diffusion of drug molecules from the implants by using the above equations. The first equation was used to calculate the diffusion coefficient in the early stage, where as the second equation was used to calculate the diffusion coefficient in the later stage.

Content uniformity

The prepared implants were powdered and finely powdered implants were passed through sieve # 85/120. The powder retained on the sieve # 120 were taken for content uniformity studies. A weight of powder equivalent to 20 mg of the drug was taken in a 100 ml standard volumetric flask. To this 75 ml of 0.1 N HCl solution was added and kept over night. The volume was made upto 100 ml with 0.1 N HCl solution. The final solution was filtered using whatmann filter paper. From this 10 ml was pipetted out into a 100 ml standard volumetric flask and made upto the volume with 0.1 N HCl solution and estimated for drug content.

Thickness of the implants

Drug loaded implants were used for thickness measurement with the help of screw gauge; average thickness of three implants were taken.

Tensile strength of the implants[20]

Tensile strength of implants was determined with Hounse Field Universal testing machine for blank implants. It consists of two loaded grips. The upper one was movable and the lower one was fixed. The test implant of specific size (8 *1 cm^2) were fixed between these cell grips and force was gradually applied till the

film breaks. The tensile strength was calculated from the dial reading.

***In vitro* drug release studies**[1,2,18]

The *in vitro* release of CDDP from the implants was carried out in screw-capped vials containing 20 ml phosphate buffer pH 7.4 (PBS). The vials were placed in an incubator shaker bath at 37 ± 1^0 C at a speed setting of 25 cycles per min. Samples were withdrawn at different time (24 hr) intervals, filtered and analyzed for drug content spectrophotometrically at 302 nm. The release data was studied using the Peppas equation to study the method of diffusion of the drug from the implants. The diffusion coefficient was calculated to measure the release rate of drug molecule from the implants.

Mathematical model fitting of obtained drug release data

The *in vitro* and *in vivo* release studies data was fitted in to various mathematical models to determine which one is the best-fit model. The various parameters 'n' the time exponent, 'k' the release constant and 'R' the regression coefficient, were also calculated.

***In vitro* Degradation Studies**[7,9,22,23,24]

The implants were placed in 10 ml phosphate buffer (pH 7.4, 37 ^{0}C) containing lysozyme enzyme (1mg/ml). The PBS was changed for all the samples every day. Implants were taken out at 7, 14, 21 and 28 days washed with distilled water and air dried for 72 h. The resulting dry weights were recorded. The mass loss of the samples was determined by gravimetry.

Stability studies of the optimized formulations

Stability is defined as the ability of particular drug or dosage form in a specific container to remain with its physical, chemical, therapeutic and toxicological specifications. The optimized formulation of Cisplatin implants (F-V) was selected for the stability studies.

Optimized formulation (F-V) were packed in screw capped bottles and study was carried out by keeping at

. 25^o± 2^{o}C and 60 ± 5% RH.

. 40^o± 2^{o}C and 75 ± 5% RH.

The duration of study was for a period of 90 days. Samples were withdrawn on 0th, 15th, 30th, 45th, and 90th days and were analyzed for drug content.

RESULTS AND DISCUSSION

FT-IR analysis[2,14]

Cisplatin and optimized formulation were subjected for FT-IR spectroscopic analysis, to ascertain any interaction between the drug and polymers exists. The FT-IR spectra obtained are given in Figure 15 & 16. The characteristic peaks of the pure drug were compared with the peaks obtained for formulation and are given in Table-4. The results indicated that there is no interaction between the cisplatin and the polymers used.

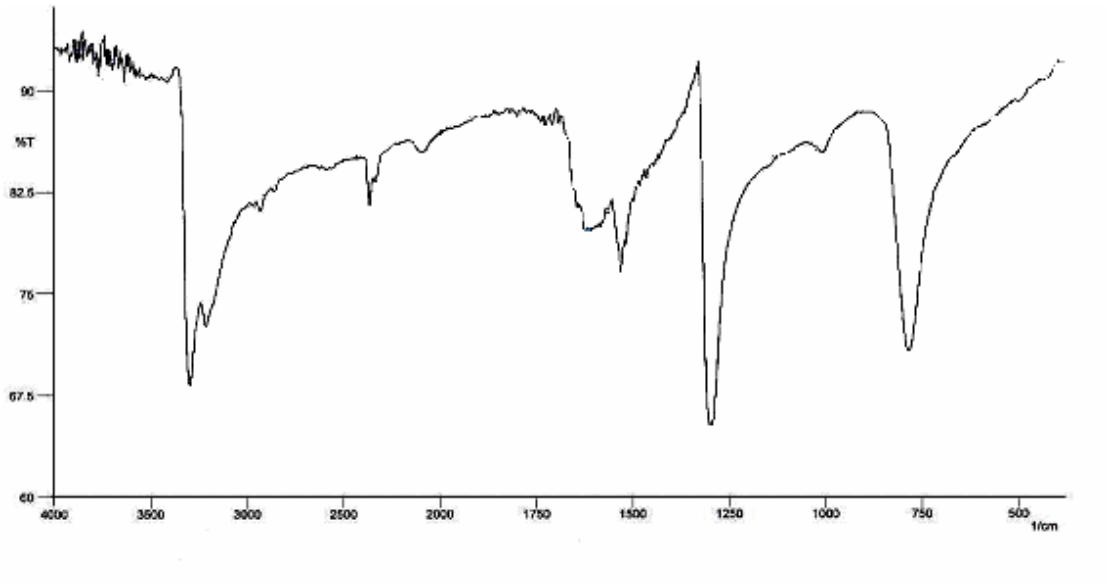

Fig 1: FTIR Spectra of Pure Cisplatin.

Fig 2: FTIR Spectra of formulation containing cisplatin.

Differential scanning calorimetry

In order to investigate the possible interactions between the drug and polymers used, differential scanning calorimetric studies were carried out. DSC thermogram of the formulation was compared with the DSC thermogram of the pure drug. The DSC thermograms obtained are reported in Figure 17 & 18. Pure Cisplatin displayed a sharp endothermic peak at 270 ^{o}C corresponding to its melting point and a similar peak was not observed in the formulation. Which may be indicative of cisplatin existing in amorphous form in the formulation.

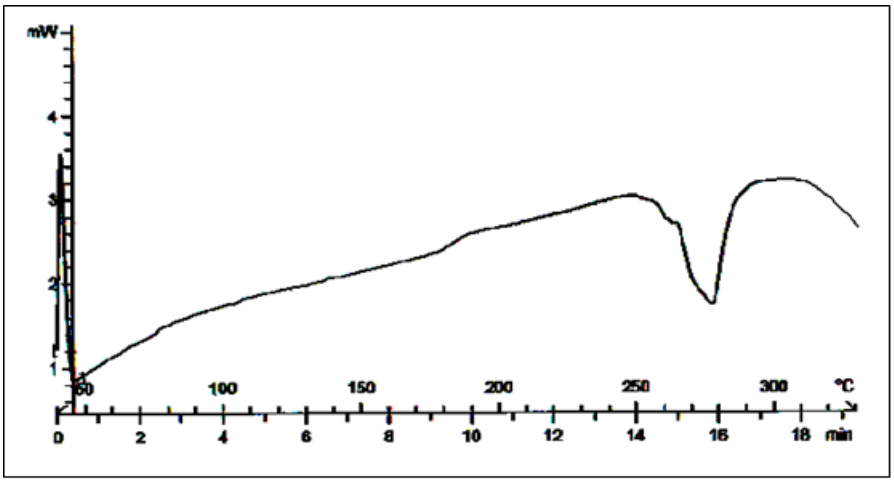

Fig 3: DSC Thermogram of pure drug.

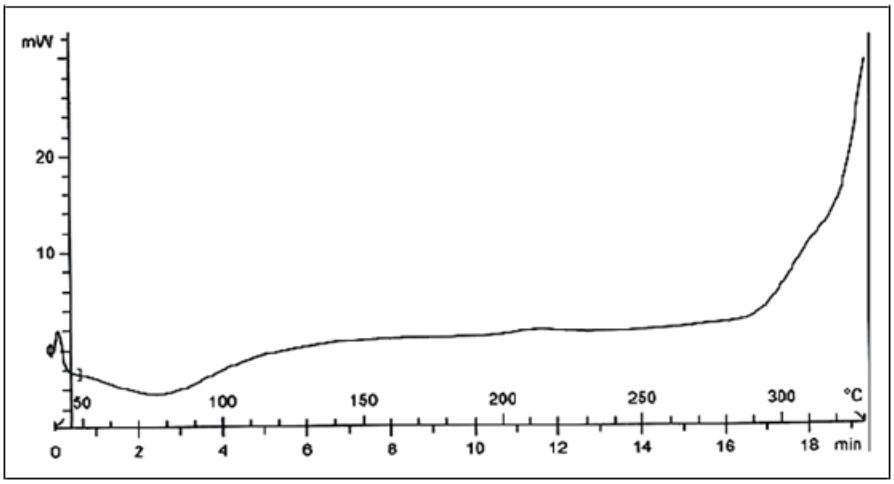

Fig. 4: DSC Thermogram of Formulation.

Scanning electron microscopy

SEM was carried out to observe the surface morphology, texture, porosity and cross section of the implants. In this study the morphological characteristics and the cross section of the implants before and after swelling were carried out.

From the SEM it was observed that, there were many dense layers in the cross section of the implant before and after swelling, on the other hand the surface of the dry hydrogels were microporous and the extent of porosity depended on the concentration of cross linker. Formulation F4 was found to be microporous due to less amount cross linker and F5 was found to be non porous due to increased concentration of crosslinker. Scanning electron micrographs obtained are given in Figures 19-22.

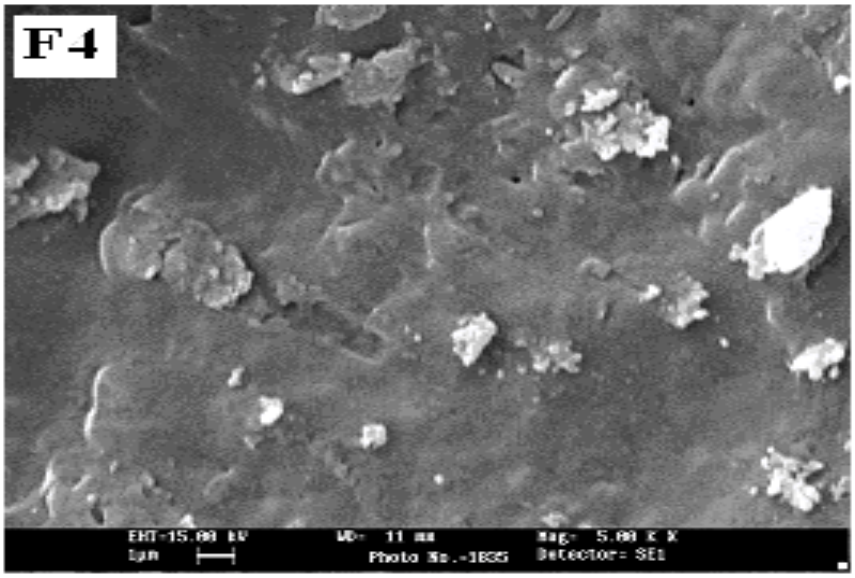

Fig 5: Surface view of the implant.

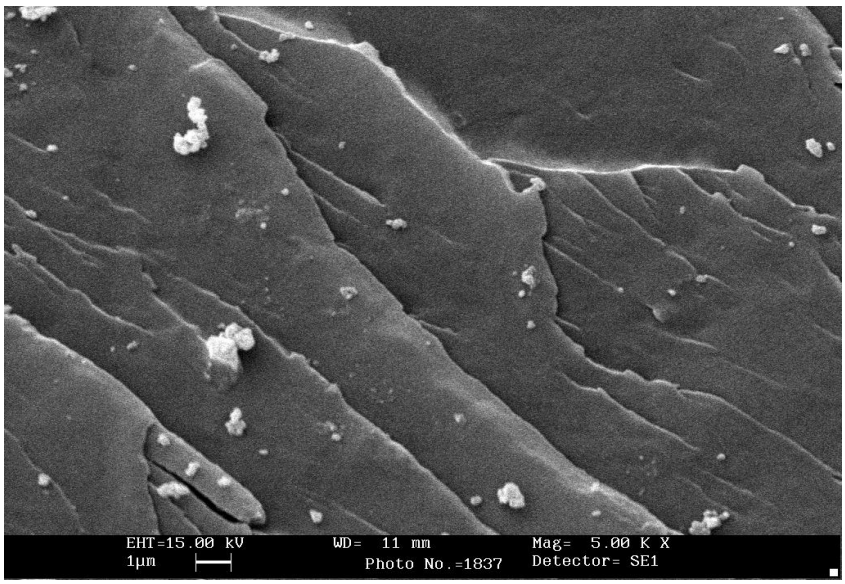

Fig 6: Cross section of implant before swelling.

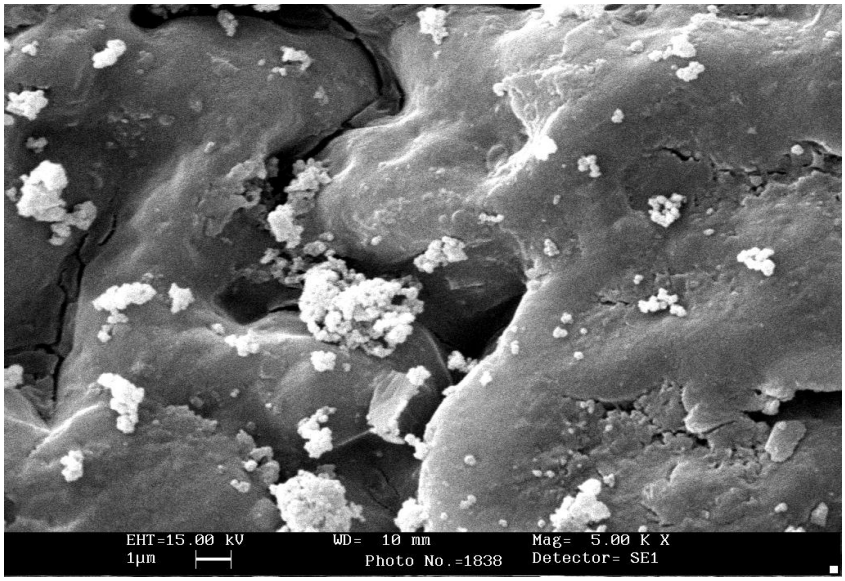

Fig 7: Cross section of implant after swelling.

Swelling studies[1,18,21,30]

The swelling studies of the implants were carried out in 7.4 pH phosphate buffer solution. The results of the swelling studies are shown in Table 5 and the graph is represented in Figure 23. Swelling depends upon the extent of cross-linking; at lower cross-linking density the network is loose with greater hydrodynamic free volume, as a result the chains can accommodate more of the solvent molecules resulting in higher swelling.

Since the release of CDDP is affected by swelling, the effect of MMA and EGDMA concentration on the swelling of hydrogels was studied. Increasing MMA content decreased the hydrophilicity of HEMA resulted in decreased swelling of formulation F3 and extent of swelling also depended on the concentration of crosslinker added in the formulation. Increasing EGDMA content decrease in the percentage of swelling, this could be attributed to the greater extent of crosslinking between the monomers which lead to decreased penetration of swelling media into the formulation F5 and swelling was found to be more in F4 due to less concentration of crosslinker. Increased swelling of F4 was found due to less amount of crosslinker. The swelling studies in different pH solution shown in (Fig 23) the n value was > 0.5 for all the hydrogel indicating Non Fickian diffusion mechanism.

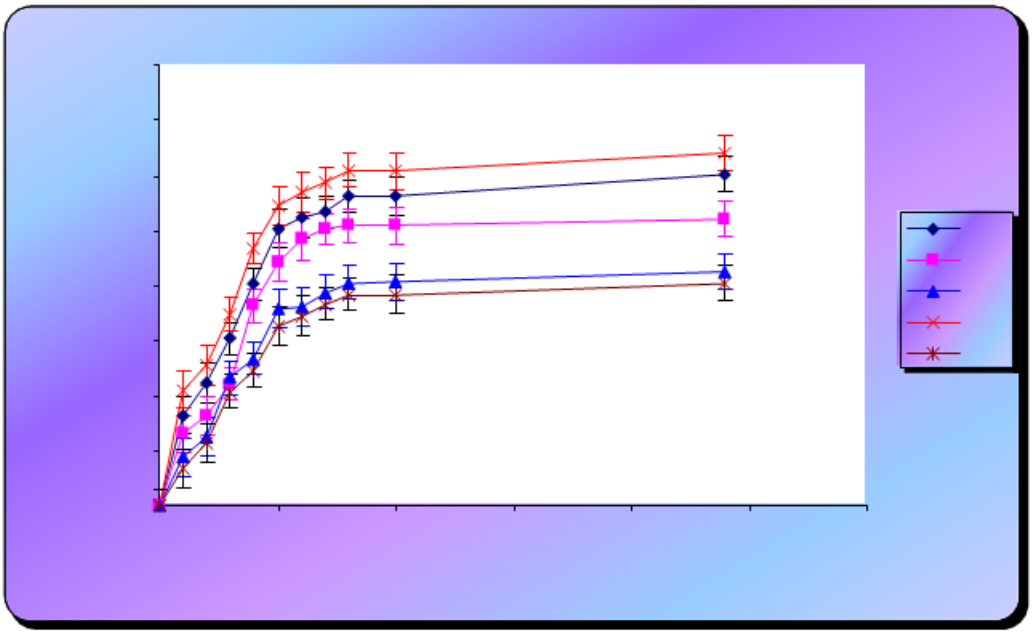

Fig 8: Graph showing percentage swelling in pH 7.4 solution.

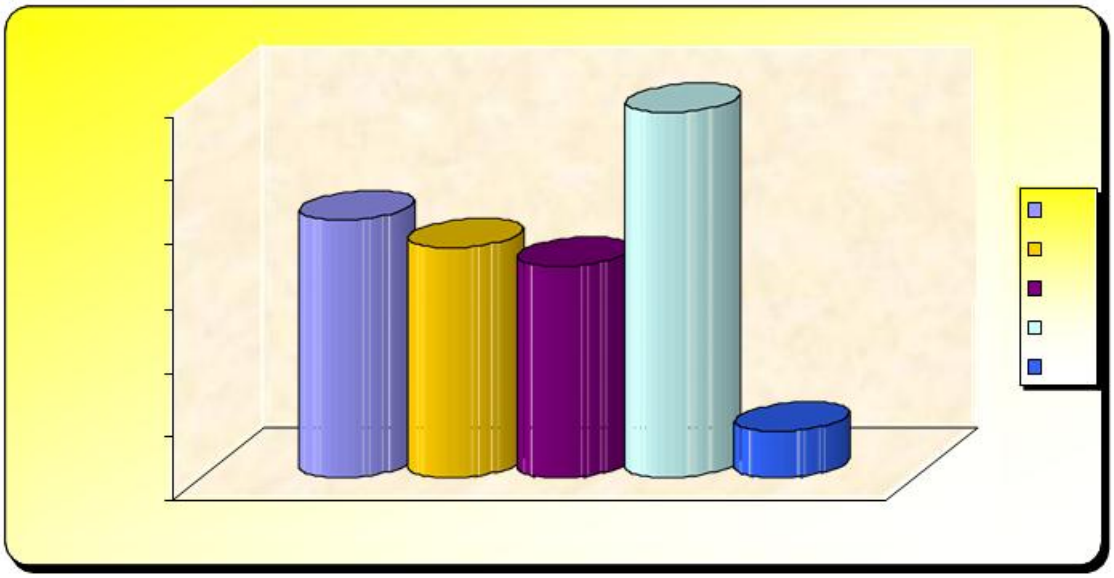

Fig 9: Diffusion coefficient for swelling studies of formulations F I to F V.

Content uniformity

The test for content uniformity was carried out to ascertain that the drug is uniformly distributed in the formulation. From the results obtained, it can be inferred that, there was a uniform distribution of CDDP in the implants and the deviation was in the acceptable limits.

Table 2: Content Uniformity data of F-I to F-V implant formulations.

Formulation Code	Trial 1	Trial 2	Trial 3	Average mean ± S.D*
F1	9.61	9.84	9.72	9.72 ± 0.12
F2	9.93	9.71	9.45	9.70 ± 0.24
F3	9.76	9.73	9.85	9.78 ± 0.06
F4	9.66	9.60	9.81	9.69 ± 0.11
F5	9.90	9.87	9.85	9.87 ± 0.03

*Standard Deviation n = 3.

Thickness

Drug loaded implants were tested for thickness and the results are given in the Table 8. From the result it was inferred that F-IV has least thickness where as the F-V has a highest thickness since it contain high concentration of cross-linking agent.

Table 3: Thickness data of F-I to F-V implant formulations.

Formulation Code	Trial I	Trial II	Trial III	Average Mean ± SD*
F I	0.631	0.625	0.641	0.632 ± 0.01
F II	0.640	0.632	0.653	0.642 ± 0.01
F III	0.641	0.625	0.624	0.613 ± 0.01
F IV	0.530	0.521	0.542	0.531 ± 0.01
F V	0.692	0.681	0.690	0.687 ± 0.01

*Standard Deviation n = 3.

Tensile strength studies[20]

Tensile strength was determined using Hounse Field universal testing machine for implants (without drug). The average of the three determinations was taken.

The results indicate that the tensile strength of the implants decreases with decrease in concentration of the EGDMA, where as the tensile strength of the implants increase with increase in the cross-linking agent concentration. The formulation F-V shows the highest tensile strength where as the formulation F- IV shows the least tensile strength.

Table 4: Results of tensile strength for F-I to F-V formulations.

Sl. No.	Formulation Code	Tensile Strenght Kg per mm^2 mean SD*
1	F I	5.4 ± 0.6
2	F II	4.3 ± 0.8
3	F III	3.2 ± 0.2
4	F IV	7.3 ± 0.6
5	F V	2.5 ± 0.4

***Standard deviation n=3**

***In vitro* release studies**[1,3,7,8,9,10,34]

The *in vitro* release studies were carried out for all the formulations in phosphate buffer saline pH 7.4 (PBS) for about four weeks.

It was observed from the results that the release of cisplatin from formulations F I to F V has varied. F IV has shown 100 % release at the end of 18th day, where as F I 92 % (21 days), F II 100% (27 days), F III 97 %(30 days), FV 89 % (30 days) This difference could be attributed to the difference in concentration of cross linker used in the formulations. F IV had lesser concentration of crosslinker (EGDMA), swelled rapidly resulted in higher release of drug compared to F V which had a higher content of crosslinker. The release mechanism was studied using Koresmeyer Peppas equation. The various parameters ëní, the time exponent, ëkí, the release constant and ëRí, the regression coefficient were calculated. The ëní value is in the range of 0.82-0.89 indicating anomalous behaviour (non-Fickian release, relaxation controlled).

From the results of diffusion coefficient studies it was observed that, the diffusion coefficient in the early stage is in the range of 4.3+ 10^{-6} to 8.0 +10^{-6} cm^2 min^{-1}, and later it was found to be in the range of 3.2 + 10^{-8} to 6.0 +10^{-8} cm^2 min^{-1}. The formulation F-V and F-III shows least diffusion coefficient, where as the F-IV formulation shows highest diffusion coefficient.

Table 5: *In vitro* release data of Cisplatin from different formulations.

Sl. No.	Time in Days	Percent Release Mean ± SD*				
		F I	F II	F III	F IV	F V
1	3	15.50 ± 4.3	14.45 ± 4.7	11.15 ± 3.4	21.60 ± 4.3	9.9 ± 3.4
2	6	23.15 ± 4.0	20.40 ± 4.2	19.22 ± 5.2	32.36 ± 3.3	20.44 ± 2.9
3	9	33.36 ± 5.2	29.39 ± 3.2	24.48 ± 1.4	50.82 ± 2.8	24.49 ± 3.8
4	12	51.18 ± 2.4	40.05 ± 3.0	34.13 ± 2.1	68.24 ± 1.3	33.69 ± 5.1
5	15	59.78 ± 3.8	52.91 ± 5.1	44.54 ± 3.8	83.59 ± 4.1	38.55 ± 4.3
6	18	72.49 ± 1.2	63.36 ± 5.3	54.69 ± 1.4	100.39 ± 3.4	47.96 ± 5.6
7	21	92.64 ± 3.2	78.68 ± 4.4	61.55 ± 4.2	-	56.0 ± 3.1
8	24	-	92.18 ± 2.1	69.66 ± 1.8	-	65.2 ± 2.4
9	27	-	100 ± 3.1	85.92 ± 4.1	-	79.1 ± 2.3
10	30	-	-	97.30 ± 0.13	-	89.0 ± 0.4

*Standard Deviation n = 3

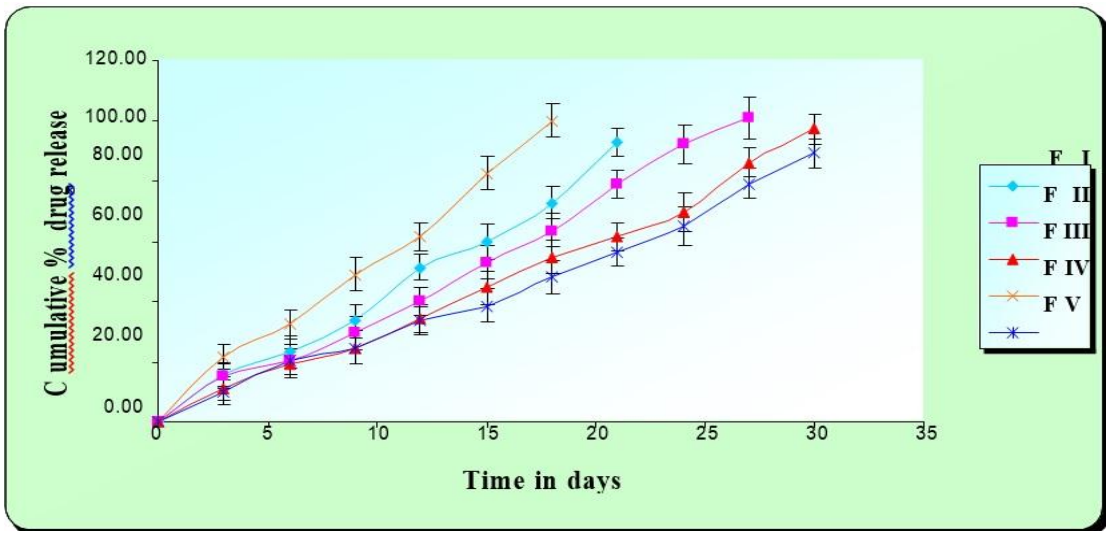

Fig 26: Graph showing the *in vitro* release profile of Cisplatin.

Effect of monomer concentration on drug release

The results indicate that the release of drug molecules from the hydrogel based implant depends on the monomer concentration in the formulation. Methylmethacrylate is a hydrophobic monomer which decreases the drug release with increase in concentration by decreasing the hydrophilicity of the hydrophilic monomer 2- hydroxyethyl methylmethacrylate. It was found that the release in formulation F3 was very much less compared to F1 and F2 due to high concentration of MMA in F3. The release profile of F1-F3 was shown in (Fig: 29).

Effect of EGDMA on drug release

The results indicate that the release of drug molecules from a hydrogel based implant depends on the characteristics of the polymer network, such as the chemical structure of the polymer, the network structure, pore size and extent of crosslinking and the release conditions, etc the drug release rate decreased with increase in EGDMA content in the hydrogel which might be due to greater extent of crosslinking between the monomer units and form a tight junctions which decrease the penetration of dissolution media into the formulation and decreases the drug release. The release of drug in the formulation F4 was high due to lesser amount of EGDMA then the F I and F5 showed less release due to high content of EGDMA in the hydrogel.

CONCLUSION

This study illustrates a novel method of drug delivery for cancer chemotherapy, where the drug is incorporated in the implants made of HEMA & MMA which can be placed directly at the cancer site to achieve controlled release of drug and attain effective treatment with reduced side effects.

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