BIOACTIVE GRAFT CO-POLYMER FOR THE TREATMENT OF
BREAST CANCER

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ABSTRACT

Cancer is one of the most researched areas nowadays due to the increasing incidences of cancer patients and unavailability of cheap and highly efficient drugs. Cancer is the uncontrolled proliferation of the body’s own cell. The cancer cells are dedifferentiated and lose their normal function. Cancer may be of two types metastatic and benign, out of which metastatic cancer is serious form. We are aware of the fact that the cancer cells differ from normal cells in many aspects. One such aspect is pH of cancer cells. The cancer cell has a pH lower than that of the normal cells of the body. In this research, we have prepared target-specific and pH-sensitive bioactive polymer using a combination of different natural polymer like polyvinyl alcohol, sodium alginate in a different ratio. Since, these polymers are pH sensitive, they will release the loaded drug only at the cancer site thus reducing the various side effects of the drug and drug toxicity.

Keywords: Breast cancer; anticancer; bioactive polymers; pH sensitive polymer

INTRODUCTION

Cancer is a multi-step process in which cells undergoes metabolic and behavioral changes, leading them to proliferate in an excessive and untimely way. These changes arise through modifications in mechanisms that control cell proliferation and lifespan, relationships with neighboring cells, and capacity to escape the immune system. Modifications that lead to cancer include genetic changes that modify the DNA sequence. Another way to change the programme of cells is to modify the conformation of chromatin, the structure that wraps up DNA and regulates its access by DNA reading, copying and repair machineries. Such changes are called “epigenetic”.
Carcinogens like ionising radiation (X Rays, UV light), chemicals – tar from cigarettes and virus infection – papilloma virus can be responsible for cervical cancer. Hereditary predisposition – Some families are more susceptible to getting certain cancers. Breast cancer is always caused by a genetic abnormality (a “mistake” in the genetic material). However, only 5-10% of cancers are due to an abnormality inherited from your mother or father. About 90% of breast cancers are due to genetic abnormalities that happen as a result of the aging process and the “wear and tear” of life in general.

Nowadays biological products are used to treat any disease to avoid side effects. Polymers are used as a biological product. Single-layer polymer doesn’t show the desired effect, therefore, alteration with their chemical structure is done. The addition of monomer (branches) to the main linear chain (backbone) is called grafting or the obtained product is known as graft copolymer. Branched molecular structures are known as graft copolymer. The addition of monomer (side chains) are random. Amphiphilic graft copolymer, solubility plays a very important role in emulsification, solubility of monomer (side chain or graft) are different from the main linear chain (backbone). The addition of graft on the polymeric chain is considered as chemical modification. In reference to the branched structure of graft copolymer, graft and backbone are of the same chemical nature. The application of comb-like polymers are the methods which are used in graft copolymer. The name graft should be given after the name of backbone polymer according to international nomenclature rule. The word graft indicates the structure of the molecules. Thus poly(styrene-graft-isoprene) means that a polystyrene backbone carries polyisoprene grafts; poly[styrene-graft-(styrene-co-isoprene)] indicates that the grafts are themselves a random copolymer of styrene and isoprene. Sometimes is difficult to understand which polymer is the backbone and which constituents the graft, therefore, nomenclature is not always obeyed. The fraction of monomer units of the backbone which carry a graft is called grafting degree, this term is commonly used in grafting technique.

**METHODS**

**OBJECTIVE OF THE EXPERIMENT:** To synthesize and characterize some graft copolymers.
**METHODOLOGY:**

The method utilized for preparing the graft co-polymer is **microwave irradiation** method.

**Method of preparation of Sample 1:**

1. The sample 1 was prepared by taking two polymers, sodium alginate, and gelatin in the ratio 1:1.
2. 150mg each of both these polymers was weighed and added in small amounts to a beaker containing distilled water just enough to dissolve the polymer, a magnetic bead was placed into it and it was then kept on a magnetic stirrer maintained at a particular temperature.
3. 100mg of acrylamide was added slowly in small amounts followed by the addition of 25 mg of ammonium persulphate (APS) slowly to the beaker.
4. The beaker was then placed in an ice bath for 1 minute and again in the microwave for 1 minute at a power of 300w.
5. This cycle was repeated until the graft copolymer was formed.
6. The graft copolymer formed was precipitated using acetone and then it was filtered.

**Method of preparation of Sample 2:**

1. Two polymers, PVA (Polyvinyl alcohol) and sodium alginate were taken in the ratio of 1:1.
2. 150mg of each of these polymers were added slowly in small amounts in a beaker containing distilled water just enough to dissolve the polymer and was then placed on a magnetic stirrer along with a magnetic bead maintained at a particular temperature.
3. 100mg of acrylamide was added slowly in small amounts followed by the addition of 25 mg of ammonium persulphate (APS) slowly to the beaker.
4. The beaker was then placed in an ice bath for 1 minute and again in the microwave for 1 minute at a power of 300w.
5. This cycle was repeated until the graft copolymer was formed.
6. The graft copolymer formed was precipitated using acetone and then it was filtered.
Method of preparation of Sample 3

1. Two polymers, PVA (Polyvinyl alcohol) and gelatin were taken in the ratio of 1:1.

2. 150mg of each of these polymers were added slowly in small amounts in a beaker containing distilled water just enough to dissolve the polymer and was then placed on a magnetic stirrer along with a magnetic bead maintained at a particular temperature.

3. 100mg of acrylamide was added slowly in small amounts followed by the addition of 25 mg of ammonium persulphate (APS) slowly to the beaker.

4. The beaker was then placed in an ice bath for 1 minute and again in the microwave for 1 minute at a power of 300w.

5. This cycle was repeated until the graft copolymer was formed.

6. The graft copolymer formed was precipitated using acetone and then it was filtered.

The graft copolymers obtained were characterized using infra-red spectrophotometry and scanning electron microscopy.

RESULTS AND DISCUSSION:

SCANNING ELECTRON MICROSCOPE (SEM) OBSERVATION

The prepared samples were dried. Then size-reduction prepared samples was done in mortar and pestle. The sample was observed under interior Scanning electron microscopy (Jeol, JSM-6390LV).

SAMPLE 1: Sodium alginate and Gelatin
Fig 1: SEM of Sodium Alginate and Gelatin at different magnification (250X, 500X, 1000X, 2000X)

SAMPLE 2: Polyvinyl alcohol (PVA) and Sodium alginate
Fig 2: SEM of PVA and Sodium Alginate at different magnification (100X, 250X, 500X, 1000X).

SAMPLE 3: Polyvinyl alcohol (PVA) and Gelatin
Fig 3: SEM of PVA and Gelatin at different magnification (100X, 250X, 500X, 1000X, 2000X).

INFRARED SPECTROSCOPIC ANALYSIS

SAMPLE 1: Sodium alginate and Gelatin

Fig 4: FTIR Spectrum of Grafted copolymer MR-I (Gelatin + Sodium alginate) – S11
Table 1: FTIR (IR bands and Groups)

Sodium Alginate + Gelatin – Physical Mixture

| IR Bands  | GROUPS                        |
|-----------|-------------------------------|
| 3540 cm\(^{-1}\) | O-H                           |
| 3379 cm\(^{-1}\) | CONH                          |
| 2897 cm\(^{-1}\) | C-H                           |
| 2140 cm\(^{-1}\) | C≡C, C≡N, N=C=O, N=C=S, N=C=N |
| 1508 cm\(^{-1}\) | C=O                           |
| 1371 cm\(^{-1}\) | C-F                           |
| 1129 cm\(^{-1}\) | C-O-C                         |
| 1003 cm\(^{-1}\) | C-O-C                         |
| 774 cm\(^{-1}\)  | C-CL                          |
| 613 cm\(^{-1}\)  | C-BR                          |

S11 - Graft

| IR Bands  | GROUPS                        |
|-----------|-------------------------------|
| 3815 cm\(^{-1}\) | N-H                           |
| 3713 cm\(^{-1}\) | N-H                           |
| 3024 cm\(^{-1}\) | C-H                           |
| 2335 cm\(^{-1}\) | C≡C, C≡N, N=C=O, N=C=S, N=C=N |
| 1761 cm\(^{-1}\) | C=O                           |
| 1508 cm\(^{-1}\) | C=O                           |

INTERPRETATION:

- The presence of a sharp peak at the wavelength of 3724cm\(^{-1}\) may be due to N-H present in gelatin.
- The presence of a sharp peak at the wavelength of 3035cm\(^{-1}\) may be due to C-H present in gelatin.
- The presence of a sharp peak at the wavelength of 2357cm\(^{-1}\) may be due to C≡N or C≡C or N=C=S or N=C=N groups present in both gelatin and sodium alginate.
- The presence of a sharp peak at the wavelength of 1761cm\(^{-1}\) may be due to the C=O group present in both gelatin and sodium alginate.
- The presence of a sharp peak at the wavelength of 1508cm\(^{-1}\) may be due to the C=O group present in gelatin.
SAMPLE 2: Polyvinyl alcohol (PVA) and Sodium alginate

Fig 5: FTIR Spectrum of Grafted co-polymer MR-II (Sodium alginate + Polyvinyl chloride) – S21

Table 2: FTIR (IR bands and Groups)
PVA (Polyvinyl alcohol) + Sodium Alginate – Physical Mixture

| IR Bands     | GROUPS                                      |
|--------------|---------------------------------------------|
| 3379 cm⁻¹    | CONH                                       |
| 2908 cm⁻¹    | C-H                                        |
| 2140 cm⁻¹    | C≡C, C≡N, N=C=O, N=C=S, N=C=N              |
| 1623 cm⁻¹    | C=O                                        |
| 1374 cm⁻¹    | C-F                                        |
| 1141 cm⁻¹    | C-O-C                                      |
| 1015 cm⁻¹    | C-O-C                                      |
| 785 cm⁻¹     | C-CL                                       |
| 613 cm⁻¹     | C-BR                                       |
S21 - Graft

| IR Bands     | GROUPS                                      |
|--------------|---------------------------------------------|
| 3575 cm⁻¹    | O-H                                        |
| 2771 cm⁻¹    | C≡C                                        |
| 2346 cm⁻¹    | C=C                                        |
| 2117 cm⁻¹    | C≡C, C≡N, N=C=O, N=C=S, N=C=N              |
| 1761 cm⁻¹    | C=O                                        |
| 1371 cm⁻¹    | C-F                                        |
| 1129 cm⁻¹    | C-O-C                                      |
| 1015 cm⁻¹    | C-O-C                                      |

INTERPRETATION:

- The presence of a sharp peak at the wavelength of 3575 cm⁻¹ may be due to the –OH group present in sodium alginate.
- The presence of a sharp peak at the wavelength 2117 cm⁻¹ may be due to C≡C or C≡N or N=C=O or N=C=S or N=C=N groups present in sodium alginate.
- The presence of a sharp peak at the wavelength 1761 cm⁻¹ may be due to the C=O group present in both PVA and sodium alginate.
- The presence of a sharp peak at the wavelength of 1371 cm⁻¹ may be due to C-X(X=halogen) group present in both sodium alginate and PVA.
- The presence of a sharp peak at the wavelength 1129 cm⁻¹ may be due to –C-O-C-group (ether) present in sodium alginate.
- The presence of a sharp peak at the wavelength of 1015 cm⁻¹ may be due to –C-O-C- group present in sodium alginate.
- Peaks were also obtained at wavelengths 2771 cm⁻¹ and 2346 cm⁻¹
SAMPLE 3: Polyvinyl alcohol (PVA) and Gelatin

Fig 6: FTIR Spectrum of Grafted co-polymer MR-III (Gelatin + Polyvinyl chloride) – S31

Table 3: FTIR (IR bands and Groups)
PVA (Polyvinyl alcohol) + Gelatin– Physical Mixture

| IR Bands    | GROUPS |
|-------------|--------|
| 3873 cm\(^{-1}\) | N-H    |
| 3747 cm\(^{-1}\) | N-H    |
| 2346 cm\(^{-1}\) | C=C    |
| 1531 cm\(^{-1}\) | C=O    |
| 1371 cm\(^{-1}\) | C-F    |
| 1189 cm\(^{-1}\) | C-O-C  |
| 785 cm\(^{-1}\)  | C-CL   |
S31 - Graft

| IR Bands       | GROUPS |
|----------------|--------|
| 3815 cm\(^{-1}\) | N-H    |
| 3713 cm\(^{-1}\) | N-H    |
| 3024 cm\(^{-1}\) | C-H    |
| 2335 cm\(^{-1}\) | C=C    |
| 1761 cm\(^{-1}\) | C=O    |
| 1508 cm\(^{-1}\) | C=O    |

INTERPRETATION:

- The presence of a sharp peak at the wavelength 3815 cm\(^{-1}\) may be due to the N-H group present in PVA.
- The presence of a sharp peak at the wavelength 3713 cm\(^{-1}\) may be due to the N-H group present in gelatin.
- The presence of a sharp peak at the wavelength 3024 cm\(^{-1}\) may be due to C-H present in both gelatin and PVA.
- The presence of a sharp peak at the wavelength 1761 cm\(^{-1}\) may be due to C=O present in both gelatin and PVA.
- The presence of a sharp peak at the wavelength of 1508 cm\(^{-1}\) may be due to C=O present in both gelatin and PVA.
- Sharp peak was also seen at the wavelength of 2335 cm\(^{-1}\).

**Testing the swelling index of different polymers**

**Preparation of buffer solution:**

- For preparation of 0.2M KH\(_2\)PO\(_4\), 13.609 gm of it was weighed and dissolved in 500 ml volumetric flask. From it 25 ml of KH\(_2\)PO\(_4\) into 100 ml of volumetric flask.
- For preparation of 0.2M NaOH, 4.00 of it was weighed and transferred to 500 ml volumetric flask.
Preparation of different pH solutions

| Sl. No. | pH  | KH$_2$PO$_4$ (ml) | NaOH (ml) |
|---------|-----|-------------------|------------|
| 1       | 6.4 | 25                | 5.80       |
| 2       | 6.6 | 25                | 8.20       |
| 3       | 6.8 | 25                | 11.20      |
| 4       | 7.0 | 25                | 14.55      |
| 5       | 7.2 | 25                | 17.35      |
| 6       | 7.4 | 25                | 19.55      |
| 7       | 7.6 | 25                | 21.20      |
| 8       | 7.8 | 25                | 22.25      |

Swelling properties of polymer MR-I (Gelatin + Sodium alginate)

| Sl. No. | Polymer (MR-I) pH | Particle size of polymer before swelling (mm) | Time taken to swell (min) | Percentage change in Particle size of polymer after swelling |
|---------|-------------------|-----------------------------------------------|---------------------------|-----------------------------------------------|
| 1       | 6.4               | 10.0                                          | 1.30                      | 50.00                                         |
| 2       | 6.6               | 10.2                                          | 1.25                      | 68.00                                         |
| 3       | 6.8               | 9.9                                           | 0.29                      | 105.00                                        |
| 4       | 7.0               | 10.0                                          | 0.30                      | 98.00                                         |
| 5       | 7.2               | 10.1                                          | 0.36                      | 95.00                                         |
| 6       | 7.4               | 10.0                                          | 0.32                      | 102.00                                        |
| 7       | 7.6               | 9.8                                           | 1.00                      | 75.00                                         |
| 8       | 7.8               | 10.1                                          | 1.02                      | 64.00                                         |

Swelling properties of polymer MR-II (Sodium alginate + Polyvinyl chloride)

| Sl. No. | Polymer (MR-II) pH | Particle size of polymer before swelling (mm) | Time taken to swell (min) | Percentage change in Particle size of polymer after swelling |
|---------|-------------------|-----------------------------------------------|---------------------------|-----------------------------------------------|
| 1       | 6.4               | 9.5                                           | 1.15                      | 45.00                                         |
| 2       | 6.6               | 9.8                                           | 1.22                      | 55.00                                         |
| 3       | 6.8               | 10.2                                          | 0.28                      | 109.00                                        |
| 4       | 7.0               | 10.0                                          | 0.31                      | 97.00                                         |
| 5       | 7.2               | 10.0                                          | 0.33                      | 99.00                                         |
| 6       | 7.4               | 10.1                                          | 0.34                      | 104.00                                        |
| 7       | 7.6               | 9.6                                           | 1.14                      | 65.00                                         |
| 8       | 7.8               | 10.0                                          | 1.05                      | 76.00                                         |
Swelling properties of polymer MR-III (Polyvinyl alcohol + Gelatin)

| Sl. No. | Polymer (MR-III) pH | Particle size of polymer before swelling (mm) | Time taken to swell (min) | Percentage change in Particle size of polymer after swelling |
|---------|---------------------|-----------------------------------------------|---------------------------|------------------------------------------------------------|
| 1       | 6.4                 | 9.7                                           | 1.10                      | 47.00                                                      |
| 2       | 6.6                 | 9.9                                           | 1.18                      | 60.00                                                      |
| 3       | **6.8**             | **10.0**                                      | **0.29**                  | **106.00**                                                 |
| 4       | **7.0**             | **10.2**                                      | **0.32**                  | **98.00**                                                  |
| 5       | **7.2**             | **10.1**                                      | **0.34**                  | **95.00**                                                  |
| 6       | **7.4**             | **10.0**                                      | **0.36**                  | **102.00**                                                 |
| 7       | 7.6                 | 9.4                                           | 1.07                      | 62.00                                                      |
| 8       | 7.8                 | 9.6                                           | 1.09                      | 73.00                                                      |

INTERPRETATION:
1. At pH 6.8 - 7.4 the swelling of polymer is maximum therefore this mean polymer can deliver the drug at specific pH site.
2. The polymer may also show property for identification of metastatic cancer cells developing in other parts.
3. Since, the drug is site specific it will show less toxicity and dosage may also get reduced.
4. Side effects of the drug will also decrease due to this target specific property

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