ORIGINAL ARTICLE

Determination of Rate of Release of Dye from the Hydrogels using Spectrophotometer Studies

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Abstract

Introduction: The main aim of the research was to determine the rate of release of dye from the hydrogels using spectrophotometer studies. **Materials and Methods:** The gelatin (GE)-polyethylene glycol (PEG) composite hydrogels were prepared by the simultaneous method. Several combinations of GE-PEG composite hydrogels were prepared with methylene blue (MB) dye in it. The components were thoroughly mixed by stirring the solution during preparation to make them homogeneous. **Results and Discussion:** The effects of glutaraldehyde, GE, and PEG variation on dye release with different hydrogel composition samples investigated. The results showed that as the concentration of cross-linking agent increases from 5 ml to 15 ml, absorbance of the sample decreased from 0.43 to 0.42 in demineralized water and 0.47 to 0.42 in 0.1M HCl due to decrease in the pore diameter of the sample, and with increased GE composition from 2.0 g to 4.0 g, absorbance of the sample also increased. **Conclusion:** The increased amount of GE decreased the intactness of the matrix, allowing the greater release of MB. The hydrophilic nature of PEG increases the interaction with the solvent which causes swelling of the microvoids, thus resulting in the high release of MB.

Key words: Gelatin, glutaraldehyde, hydrogels, methylene blue, microvoids, polyethylene glycol, spectrophotometer

INTRODUCTION

Hydrogels are three-dimensional (3D) cross-linked structural arrangement of the polymeric materials with the ability to absorb large amounts of water while maintaining their dimensional stability.[1,2] The 3D structural integrity of hydrogels in their swollen state is maintained by either physical or chemical cross-linking.[1,3] The chemically cross-linked networks involve permanent junctions like covalent bonds, whereas the physical networks arise from either polymer chain entanglements or physical interactions such as ionic interactions, hydrogen bonds, or hydrophobic interactions. The extent of polymerization and cross-linking determines the shape and size of the gel materials. In polymeric gel, there is a long chain of polymers termed as the dispersed phase and the other components are the dispersion medium which may be the liquid like water. A gel contains near about 90–99% water molecules as a dispersion medium.[4,5] The presence of liquids in interstitial spaces of the gels makes them soft, wet, and elastic. In dry conditions, the gel is usually coiled and did not show good property.[3,6] The gels

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have the ability to undergo significant deformations because they are soft and filled with a solvent. The presence of other liquid solvents may impart the deformation properties in various shapes and sizes. These gels can expand or shrink up to 1000 times in volume. The different macromolecular structures of hydrogels include cross-linked or entangled networks of linear homopolymers, linear copolymers, block or graft copolymers, polyion–polyion or H-bonded complexes, hydrophilic networks, and interpenetrating polymer networks or physical blends.[2,5-6]

The different physical forms of hydrogels are solid molded forms (e.g., soft contact lenses), pressed powder matrices (e.g., pills or capsules for oral ingestion), microparticles (e.g., as bioadhesive carriers or wound treatments), coatings (e.g., on implants or catheters; on pills or capsules, or coatings on the inside capillary wall in capillary electrophoresis), membranes or sheets (e.g., as a reservoir in a transdermal drug delivery patch; or for two-dimensional electrophoresis gels), encapsulated solids (e.g., in osmotic pumps), and liquids (e.g., that form gels upon heating or cooling).[7]

The synthetic polymers that form hydrogels are usually obtained through chemical polymerization methods by cross-linking the materials of desirable properties. These hydrogels may have lower interfacial tension, soft and tissue-like physical properties, higher permeability to undersized molecules, and release of entrapped molecules in a controlled manner. These properties have made hydrogels a focus of exploration in different biomedical fields.[2,8]

Properties of hydrogels

The most important characteristic properties of the hydrogels that are needed to be examined before their applications are swelling behavior, mechanical properties, toxicity studies, biocompatible properties, degradation behavior, other technical features like stability and constancy in a swelling, absorption ability, rate of absorption, particle size and porosity, physical properties, non-toxic, the highest absorbency under load, maximum biodegradability, hygiene applications, and rewriting capability.[3,9-11]

Different types of hydrogels are thermoreversible hydrogels, glucose-responsive hydrogels, stimuli-responsive hydrogels, pH-responsive hydrogels, temperature-responsive hydrogels, antigen-responsive hydrogels, magnetically responsive system, and light-sensitive system ion-sensitive system.[2] In this study, we focus on the release of dyes from hydrogels.

MATERIALS AND METHODS

Hydrochloric acid (HCl) (S. dfine, India), Gelatin (GE) (Fluka [Buchs, Switzerland]), methylene blue (MB) (S. dfine, India), polyethylene glycol (PEG) (Sigma-Aldrich chemicals [USA]), glutaraldehyde (GA) (S. dfine, India). All the reagents and chemicals utilized were of analytical grades.

MB

MB, is also known as methylthioninium chloride, is a formal derivative of phenothiazine used in medicines and dye. It is a dark green powder that yields a blue solution in water. Its molecular formula is $C_{16}H_{18}ClN_3S$. MB is on the World Health Organization's list of essential medicines. As a medication, it is mainly used to treat methemoglobinemia.

PEG

PEG, also sometimes referred to as polyethylene oxide, is a condensation polymer of ethylene oxide and water. PEGs are used for biological, chemical, and pharmaceutical applications. When cross-linked into networks, PEG can have high water content, forming hydrogels. Hydrogel formation can be initiated by cross-linking PEG by either ionizing radiation or by covalent cross-linking. PEGs do not hydrolyze or deteriorate upon storage and do not support the growth of molds. The hydrophilic characteristics and biocompatibility make PEG an excellent tool for polymer-based drug delivery and biomedical applications.

GE

GE is a heterogeneous mixture of water-soluble protein of high average molecular weights present in collagen. These proteins are extracted from skin, tendons, ligaments, bones, etc. The applications of GE include coating cell culture plates to improve cell attachment for a variety of cell types. In bacteriology, GE can be used as a component of culture media for species differentiation. It is commonly used for pharmaceutical and medical applications because of its biodegradability and biocompatibility in physiological environments. It is used in food preparation and as a basis of many jellies, desert, and candies.

GA

GA is a pale-yellow liquid having a pungent and rotten-apple smell. It causes irritation in eyes, skins, and respiratory tract when comes in contact. It is used to develop cross-linking in the hydrogels.

Preparation of different formulations of hydrogels

The composition of the prepared samples is given in Table 1.

Swelling study

The maximum hydration capacity of the GE and PEG hydrogel was determined by weighing the dried sample (W_d) and then weighing the sample again after immersion in demineralized water (DMW) at room temperature for 30 min.[6] The water absorption of the sample was calculated using the following equation:

$$I_s = \frac{W_d - W_s}{W_d} \times 100$$

Where I_s is the percentage swelling index. Figure 1 shows the photographs of the (a) dried and (b) swollen hydrogels.

Dye released study

The release of dye from the hydrogels was studied by taking the fixed weight of dry hydrogel containing the dye in 100 ml DMW/HCl solution. The amount of dye released was quantitatively determined by measuring the absorbance using a spectrophotometer at a regular interval of time.

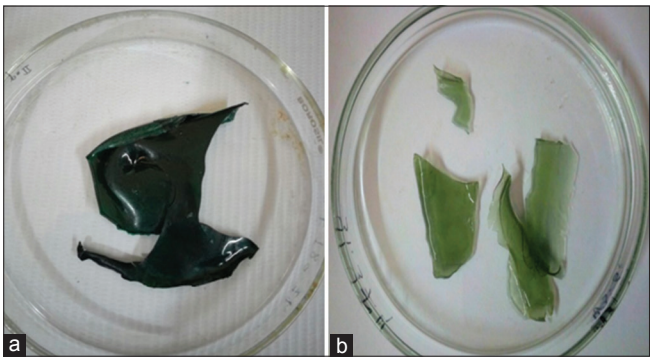

Figure 1: Swelling in hydrogel (a) before swelling (b) after swelling

Table 1: Weights of the chemicals taken in different formulations of hydrogels

Samples prepared	Components			
	GE (g)	PEG (g)	MB (1000 ppm) (ml)	GA (ml)
S.P-1	02.00	00.50	10.00	10.00
S.P-2	02.00	00.50	10.00	20.00
S.P-3	02.00	00.50	05.00	10.00
S.P-4	02.00	00.50	05.00	05.00
S.P-5	02.00	01.50	05.00	10.00
S.P-6	03.00	00.50	05.00	10.00
S.P-7	04.00	02.50	05.00	15.00

PEG: Polyethylene glycol, MB: Methylene blue, GA: Glutaraldehyde, GE: Gelatin

RESULTS AND DISCUSSION

Effects of cross-linker variation on dye release

Tables 2 and 3 determine the membrane dye releasing property due to the variations of cross-linker (5 ml, 10 ml, and 15 ml) which shows the release behavior of MB from the membrane in the hydrogel compositions S.P- 3, 4 and 7 in DMW and 0.1M HCl, respectively. The observation showed that the decrease in the absorbance of MB release through the sample membrane may be due to the contraction of the microvoids formed between the polymeric chain.[12-15]

Table 2: Dye release comparison in sample – 3, 4, and 7 in DMW (GA variation).

Time taken (min)	S.P - 4 (5 ml GA)	S.P - 3 (10 ml GA)	S.P - 7 (15 ml GA)
30	0.08	0.07	0.05
60	0.12	0.12	0.11
90	0.18	0.17	0.16
120	0.22	0.21	0.19
150	0.25	0.24	0.23
180	0.27	0.26	0.25
210	0.30	0.29	0.27
240	0.32	0.31	0.29
270	0.34	0.33	0.33
300	0.36	0.36	0.34
330	0.39	0.38	0.37
360	0.43	0.41	0.39
390	0.43	0.41	0.40
420	0.43	0.42	0.40
450	0.43	0.42	0.41
480	0.43	0.42	0.41
510	0.43	0.42	0.41
540	0.43	0.42	0.41

GA: Glutaraldehyde, DMW: Demineralized water

Table 3: Dye release comparison in sample – 3, 4, and 7 in 0.1M HCl (GA variation)

Time taken (min)	S.P-4 (5 ml GA)	S.P-3 (10 ml GA)	S.P-7 (15 ml GA)
10	0.45	0.24	0.20
20	0.47	0.38	0.29
30	0.47	0.43	0.37
40	0.47	0.43	0.41
50	0.47	0.44	0.42
60	0.47	0.44	0.42
70	0.47	0.45	0.42

GA: Glutaraldehyde

Obtained experimental results illustrate that with an increasing cross-linking agent from 5 ml to 15 ml, absorbance of the prepared sample decreased from 0.426 to 0.422 in DMW and 0.474 to 0.419 in 0.1M HCl due to decrease in the pore diameter of the sample.

Table 4: Dye release comparison of sample – 3, 6, and 7 in DMW (Ge variation).

Time taken (min)	S.P- 3 (2 gmGe)	S.P- 6 (3 gmGe)	S.P- 7 (4 gmGe)
30	0.10	0.12	0.14
60	0.17	0.18	0.20
90	0.18	0.21	0.23
120	0.20	0.22	0.25
150	0.21	0.24	0.26
180	0.22	0.23	0.26
210	0.23	0.25	0.26
240	0.23	0.25	0.26
270	0.24	0.26	0.27
300	0.25	0.26	0.27
330	0.26	0.27	0.27
360	0.26	0.26	0.27
390	0.26	0.27	0.26
420	0.27	0.26	0.26
450	0.27	0.27	0.27
480	0.27	0.27	0.27

DMW: Demineralized water

Table 5: Dye release comparison of sample – 3, 6, and 7 in 0.1M HCl (Ge variation).

Time taken (min)	S.P- 3 (2 gmGe)	S.P- 6 (3 gmGe)	S.P- 7 (4 gmGe)
10	0.10	0.12	0.27
20	0.17	0.21	0.34
30	0.22	0.27	0.38
40	0.25	0.32	0.38
50	0.27	0.34	0.38
60	0.28	0.36	0.38
70	0.29	0.36	0.38
80	0.30	0.37	0.38
90	0.31	0.37	0.38
100	0.32	0.37	0.39
110	0.34	0.38	0.38
120	0.35	0.38	0.39
130	0.35	0.38	0.39
140	0.36	0.38	0.39
150	0.36	0.38	0.38
160	0.36	0.38	0.39

Effects of GE on dye release

Tables 4 and 5 determine the effect of MB release from the Ge-PEG composite hydrogel with varying amount of GE (2 g, 3 g, and 4 g) at a fixed amount of GA and PEG in the hydrogel compositions S.P – 3, 6, and 7 in DMW and 0.1M HCl, respectively. According to the experimental result, we conclude that with increasing GE compositions from 2 g to 4 g, absorbance of the prepare sample also increased. This is due to the hydrophilic nature of GE that the interaction between MB dye and GE is high which allows them to penetrate in the matrix and facilitate the MB release. The increased amount of GE decreased the intactness of the matrix, allowing the greater release of MB.

Table 6: Dye release comparison in sample 3, 5, and 7 in DMW (PEG variation)

Time taken (min)	S.P- 3 (0.5 g PEG)	S.P- 5 (1.5 g PEG)	S.P- 7 (2.5 g PEG)
30	0.14	0.19	0.22
60	0.20	0.22	0.24
90	0.23	0.23	0.26
120	0.24	0.26	0.27
150	0.25	0.26	0.27
180	0.26	0.27	0.27
210	0.26	0.27	0.27
240	0.26	0.27	0.27
270	0.26	0.27	0.27
300	0.26	0.27	0.27
330	0.26	0.27	0.27
360	0.26	0.27	0.27

PEG: Polyethylene glycol, DMW: Demineralized water

Table 7: Dye release comparison in sample – 3, 5, and 7 in 0.1M HCl (PEG variation)

Time Taken (minutes)	S.P- 3 (0.5 g PEG)	S.P- 5 (1.5 g PEG)	S.P- 7 (2.5 g PEG)
10	0.27	0.24	0.22
20	0.34	0.33	0.29
30	0.38	0.35	0.31
40	0.38	0.36	0.32
50	0.38	0.36	0.34
60	0.38	0.36	0.34
70	0.38	0.36	0.35
80	0.38	0.36	0.35
90	0.39	0.36	0.35
100	0.39	0.36	0.36
110	0.39	0.36	0.36

PEG: Polyethylene glycol

Effects of PEG variation on dye release

Tables 6 and 7 determine the dye release behavior of compositions containing varying amounts of PEG (0.5 g, 1.5 g, and 2.5 g) with fixed GA and GE in the hydrogel compositions S.P – 3, 5, and 7 in DMW and 0.1M HCl, respectively. The observation showed that with increasing PEG compositions from 0.5 g to 2.5 g, absorbance of the prepared sample also increased. This may be due to the hydrophilic nature of PEG that the increased in the PEG amount suggested its increased interaction with the nearby solvent which causes swelling of the microvoids, thus resulting in the high release of MB.

CONCLUSION

Our study concludes that with increasing cross-linking agents from 5 ml to 15 ml, absorbance of the prepared sample decreased from 0.43 to 0.42 in DMW and 0.47–0.42 in 0.1M HCl due to a decrease in the pore diameter of the sample. The hydrophilic nature of GE that the interaction between MB dye and GE is high which allows them to penetrate in the matrix and facilitate the MB release. The increased amount of GE decreased the intactness of the matrix, allowing the greater release of MB. With increasing PEG compositions from 0.5 g to 2.5 g, absorbance of the prepared sample also increased. This may be due to the hydrophilic nature of PEG that the increased in the PEG amount suggested its increased interaction with the nearby solvent which causes swelling of the microvoids, thus resulting in the high release of MB. Hydrogel of many synthetic and natural polymers have been produced with their end-use mainly in tissue engineering, pharmaceutical, and biomedical fields. Due to their high water absorption capacity and biocompatibility, they have been used in wound dressing, drug delivery, agriculture, sanitary pads as well as trans-dermal systems, dental materials, implants, injectable polymeric systems, ophthalmic applications, and encapsulated living cells.

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CONTRIBUTION OF AUTHORS

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