Journal of Innovation in Applied Research

E-ISSN xxxx - xxxx
Available at: http://www.jiar.in

RESEARCH ARTICLE

NON-ACETYLATED HEMICELLULOSE-BASED HYDROGEL PREPARATION FOR SUSTAINED AND TARGETED DELIVERY OF DRUGS

Samit Kumar[1]*, Riya Khilwani[2] and Kamlesh Choure[2]
[1]Department of Chemistry, Faculty of Basic Science, AKS University, Sherganj Road, Satna- 485001 (M.P) India
[2]Department of Biotechnology, Faculty of Life Science and Technology, AKS University, Sherganj Road, Satna- 485001 (M.P) India
***Corresponding Author:** E-mail: samitiitr07@gmail.com

Received on: 10.05.17; **Revised on:** 20.05.17; **Accepted on:** 15.06.17

Keywords

- Hydrogels
- Hemicellulose
- Acrylic acid

ABSTRACT

Hydrogels are water-swollen, three-dimensional, cross-linked polymeric network produced by simple reaction of one or more monomers with the ability to swell in an aqueous medium. Consequently, hemicellulose-based hydrogels were prepared by the combination of hemicellulose (HC) and acrylic acid (AAc) in different ratios. Result showed that the hydrogels absorbed up to 300% of water when it is prepared by combining HC and AAc in the ratio of 1:8. This combination ratio was preferred as a suitable candidate for drug loading and release profile in near future. The prepared hydrogel may be used in the biomedical field for sustained and targeted release of drugs.

INTRODUCTION

In spite of good advancement for diagnosis and treatment, cancer is the second most common disease after cardiovascular disorders, may be responsible for maximum deaths in the world (Jemal et al, 2007). Amongst cancers, colon or colorectal cancer is the fourth most common form of cancer globally with 6, 39,000 deaths reported annually (World Health Organization, 2009). Colon is the distal part of gastrointestinal tract, extending from ileo-cecal junction to anus. Therefore, delivering of a drug to the colon is a devastating problem because the most of the drugs have been reported to be unstable in the gastric environment and are prone to absorption in the upper gastrointestinal tract (GIT). This causes lowering of drug bioavailability and reduction of their efficacy against Inflammatory Bowel Disease (IBD). Therefore, the delivery of a drug to the colon, via GIT requires protection from an undesirable release in stomach and small intestine (upper GIT) to achieve maximal pharmacological effect, while administered orally (Sinha and Kumria, 2003; Singh, 2007). As a consequence, Protection of drugs can be achieved by those potent carriers which are of non-toxic and biocompatible nature, and which can also undergo in-vivo biodegradation easily. Xylan rich-hemicellulose, one of such type of efficient carrier, may be used for colon drugs via hydrogel preparation.

Hydrogels are water-swollen, three-dimensional, cross-linked polymeric network produced by simple reaction of one or more monomers with the ability to swell in an aqueous medium (Hoare & Kohane, 2008). *Hydrogels are* a class of those polymers that can absorb

large amounts of water without dissolving it, may be due to the physical or chemical cross linkages of hydrophilic polymer chains. They mimic many of the properties of natural tissue so they are highly biocompatible. The porous or dense structure can be made by choosing the polymers and cross-linkers and altering the composition of salt concentration, pH and temperature (Ahmed, 2013).

In recent years, intelligent hydrogels have been widely studied because of their responsiveness to the environmental stimuli, such as pH, ionic strength, solvent composition, temperature and electric and magnetic fields. Furthermore, because of the excellent biocompatibility and biodegradability of natural polymers, a variety of natural polysaccharides have been used to prepare hydrogels. Hemicellulose (HC) is one of the polymers, isolated from agro-waste raw material like wheat straw (WS). It is the second most abundant renewable natural polymer compared to cellulose, exists widely in plants. Xylan is a group of hemicelluloses that are found in plant cell walls and some algae. Xylan is a polysaccharide made from units of xylose (a pentose sugar). It is almost as ubiquitous as cellulose in plant cell walls and contains predominantly β-D-xylose units. Therefore, preparation of xylan-based hydrogels towards colon targeted drug delivery system (DDS) is a potential area of application for hemicelluloses and their derivatives.

Thereby, the objective of the present study is to isolate the xylan-rich hemicellulose from agro-waste raw materials such as Wheat Straw followed by the preparation of intelligent hydrogel for colon targeted drug delivery system.

MATERIALS AND METHODS

The chemicals used throughout the experiment are of analytical grade.

Raw Material

The wheat straw sample was collected from agricultural field Locally (Stana, Madhya Pradesh, India) and it was milled into powder, and fractions passing through 40 mesh (400 μm) screens but retained on 80 mesh (177 μm) screens was collected. Sample was air-dried, homogenised in a single lot to avoid compositional differences among aliquots, and stored for compositional analysis.

Compositional Analysis

Compositional Analysis of wheat straw samples used as raw material (as the average of three replicate analyses) is shown in **Table 1**. The results are expressed as weight percent of cellulose, hemicellulose, lignin, ethanol-benzene soluble, hot water soluble and ashes by using TAPPI Standard Test Methods. Other fractions counting uronic acids, soluble lignin, acetyl groups etc., have minor importance for the purpose of this research work and thereby they are not determined.

Table 1: Fractions of Wheat Straw (as the average of three replicate analyses)

Fractions	Weight % on oven dry basis
Cellulose	46.7
Hemicellulose	25.6
Lignin	15.4
Ethanol-Benzene solubles	5.2
Hot water soluble	4.3
Ash	2.8

Isolation of Xylan-rich Hemicellulose

The non-cellulosic product such as xylan-rich hemicellulose was extracted from wheat straw an agro-waste raw material, after proximate analysis as represented in schematic diagram as shown in figure 1. For the extraction of xylan-rich hemicellulose, the wheat straw was firstly soxhleted followed by delignification to remove coloring material and lignin respectively. The resulting material contains cellulose and hemicellulose collectively known as holocellulose. The holocellulose was further treated with 2.5N of NaOH solution for 6h at room temperature to dissolve the xylan-rich hemicelluloses followed by filtration through Whatman 44 filter paper to separate the extract and residue. The extract was neutralized with 2.5N solution of acetic acid, and xylan was separated by settling down after methanol addition. Subsequently, several washing steps were performed by using methanol. Finally, the sample was filtered and dried at 60°C in vacuum oven, yielded 12 ± 1.4% (n=3) on oven dry basis. The characterization processes were carried out from the same single bulk of polymer.

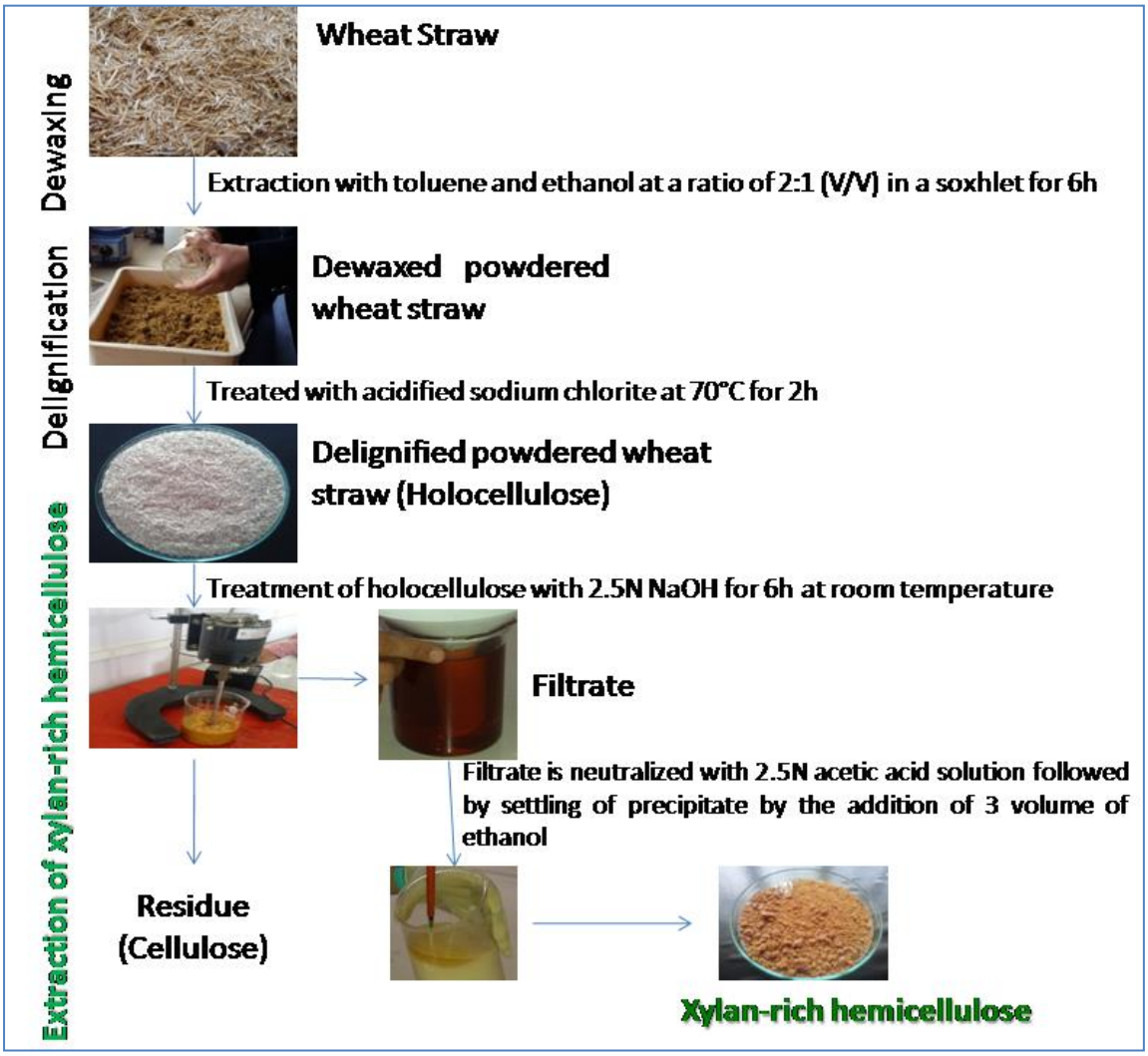

Figure 1: Schematic representation of isolation of xylan-rich hemicellulose

Preparation of Hemicellulose-based Hydrogels

The isolated xylan-rich hemicellulose was used for hydrogel preparation. For hydrogel preparation a redox initiation system such as Sodium Sulphite (Na_2SO_3) and Potassium persulphate ($K_2S_2O_8$), MBA (N, N´- methylene bis acryl amide) a cross-linker and Acrylic

Acid (AAc) based homopolymer were employed with hemicellulose. The complete experiments were performed at 50°C in a water bath as shown in schematic diagram 2.

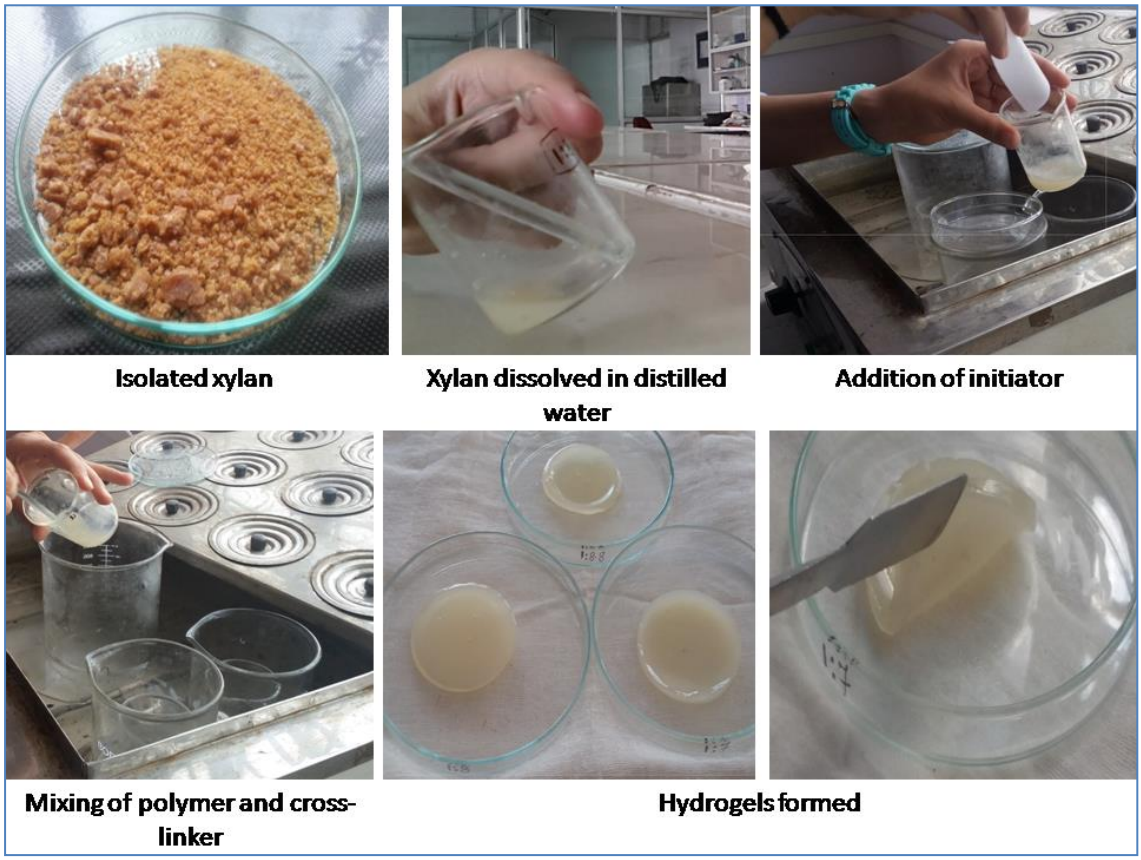

Figure 2: Schematic representation of hemicellulose based-hydrogels preparation

For hydrogel preparation hemicellulose was first dissolved in distilled water to form a solution (5% w/w) followed by the addition of redox initiator system (0.01g of $K_2S_2O_8$ and 0.04g of anhydrous Na_2SO_3). Subsequently, different amount of acrylic acid (AAc) homopolymer was added slowly and then after 5 minutes 0.024g of solubilized crosslinker (N,-N-methylene bisacrylamide) were added. The reaction system became progressively thicker until it could not be stirred. The amount of different components was added for the preparation of hydrogels is shown as in **Table 2.**

Table 2: Preparation of hydrogels were carried out in three different ratios of AAc to hemicellulose

Sample code	**Reaction condition**		
	AAc/HC	**Initiator (0.01g of Na_2SO_3 + 0.04g of $K_2S_2O_8$)**	**Crosslinker (MBA)**
Gel 1	(1.7ml/0.25 gm) 7:1	0.05g	0.024g
Gel 2	(2.0ml/0.25 gm) 8:1	0.05g	0.024g
Gel 3	(2.2ml/0.25 gm) 8.8:1	0.05g	0.024g

The prepared sample was removed, cut into cuboids (approximately 8mm long, 4mm wide and 2mm thick) and immersed in de-ionized water for 48h. The de-ionized water was refreshed every 4h to remove the impurities, especially the acrylic acid homopolymer. The swollen hydrogel samples were subsequently dried in vacuum oven at 50°C to a constant weight for further characterization.

RESULTS AND DISCUSSION

Isolation of Xylan-rich Hemicellulose from Wheat Straw

Hemicellulose was extracted from wheat straw (WS) by mechano-chemical treatment as shown in Figure of experimental method. The dewaxed sample of WS powder was delignified with acidified $NaClO_2$ solution at 70°C without degrading cellulose and hemicellulose.

$$5\ NaClO_2 + 4\ CH_3COOH \xrightarrow{70^oC} 4\ CH_3COONa + 4\ ClO_2 + NaCl + 2H_2O$$

The ClO_2 act as a bleaching agent, removing coloring material and lignin however, delignification process could not dissolve whole of the lignin. It is found that the cellulose micro-fibril in a hydrophobic matrix is physically shielded by lignin and lignin is covalently bonded with cellulose and hemicellulose, as a result, lignin could not be separated completely from celluloses and hemicelluloses.

Therefore, the delignified product was treated with 250ml of 2.5N NaOH solution to extract the xylan and simultaneously, to remove the residual lignin. Alkali treatment cleaves the β-alkyl-aryl ether bonds (β-O-4 linkages) in lignin via the formation of an epoxide intermediate with an intramolecular nucleophilic displacement of β-phenoxide by either α- or γ- alkoxide, formed from alcohol in strong base. Subsequent nucleophilic attack by hydroxide cleaves the epoxide ring and produces a tri-hydroxyl propane structure (Kumar et al., 2010). Thus the residual lignin was removed by NaOH treatment and xylan was extracted from alkaline filtrate by precipitation with 2.5N acetic acid, settling down with ethanol, several washing steps were performed using ethanol. Finally, the sample was filtered and dried at 50°C in vacuum oven. The characterization process was made from the same single bulk of polymer. The extracted hemicellulose is non-acetylated in nature. The acetyl groups present as side chain in the main chain of polymer of five membered ring of xylose are more susceptible towards alkaline treatment. Thereby, the acetyl groups are easily removed resulting as non-acetylated xylan rich-hemicellulose.

FT-IR Spectroscopy of Holocellulose and Xylan-rich Hemicellulose

The xylan-rich hemicellulose were non-acetylated in nature may also be confirmed by FT-IR Spectrophotometer. FT-IR spectroscopy is a powerful tool for studying the physico-chemical and conformational properties of polysaccharides. The most of the absorption bands of holocellulose and xylan-rich hemicellulose are similar as that of reported (Kumar et al., 2010, 2012, 2014). However, one of the absorption bands observed at a wavenumber of 1734 cm^{-1} relates to C-O stretching of carbonyl group in holocellulose which was disappeared in xylan-rich hemicellulose as shown in figure 3.

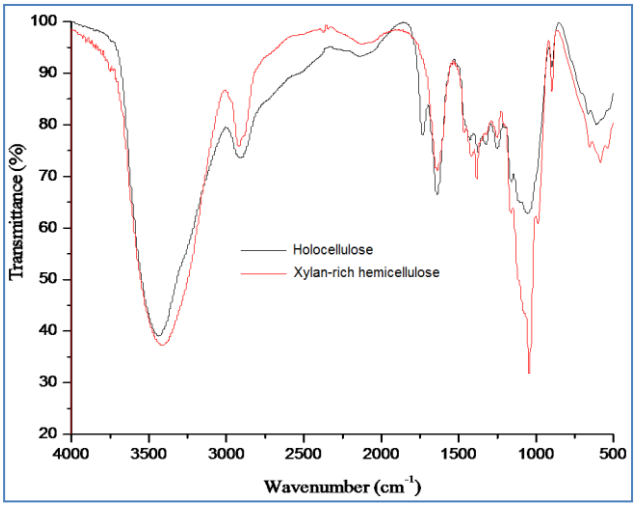

Figure 3: FT-IR spectra of holocellulose and xylan-rich hemicellulose

The disappearance of absorption band in case of xylan-rich hemicellulose is a sign of removal of acetyl groups. In other words, we can say that the obtained alkali treated product must be free of acetyl groups, called non-acetylated xylan-rich hemicellulose.

Characterization of Hydrogels

The HC/AAc ratios, the amounts of initiator and crosslinker, and the degree of AAc neutralization will affect the chemical structure and properties of the prepared hydrogels. All of these factors were varied to study the influences on the physico-chemical properties of HC-based hydrogels.

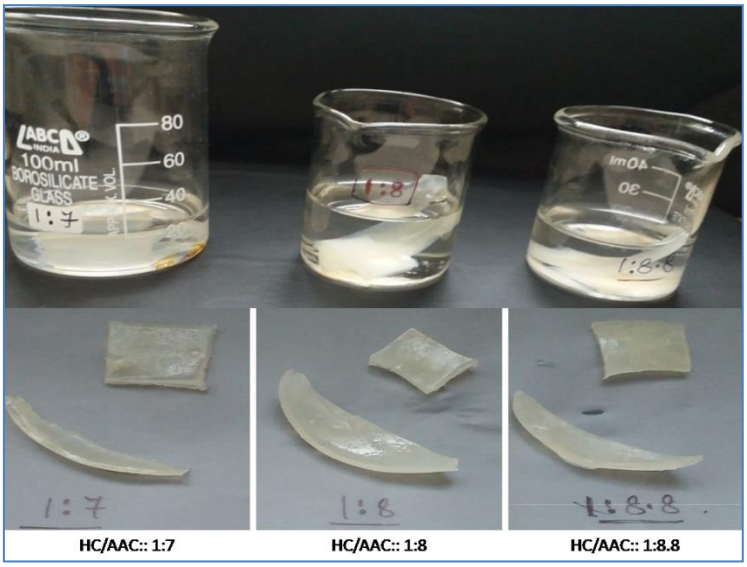

Figure 4: Schematic diagram of swelled hydrogels of three combinations of hemicellulose and acrylic acid

The cuboids cut of oven dried hydrogels were weighed and immersed into 20ml of distilled water to swell up for 24h as shown in figure 4. Subsequent to swelling, the same pieces were pulled out and placed on tissue paper to remove extra water and then weighed. The percentage of swelling behavior as per the ratios of HC to AAc is presented as in **Table 3** and **Table 4.** The swelling ratio can be calculated by the formula as below,

$$S = \frac{Wt - Wd}{Wd};$$

Where, Wd is the initial weight of the dried Hydrogel and Wt is the swelled weight of hydrogels after time‘t’.

Table 3: Swelling ratio for big hydrogel pieces

Hydrogel	Wd	Wt	Swelling ratio	% of swelling
1:7	0.15g	0.69g	3.6	360
1:8	0.40g	1.94g	3.85	385
1:8.8	0.25g	1.20g	3.8	380

Table 4: Swelling ratio for small hydrogel pieces

Hydrogel	Wd	Wt	Swelling ratio	% of swelling
1:7	0.20g	0.90g	3.5	350
1:8	0.19g	0.91g	3.8	380
1:8.8	0.26g	1.14g	3.4	340

The maximum percentage of swelling 385% and 380% were observed for big piece and small piece of hydrogel formulation. These hydrogels formulations have been prepared by the combination of hemicellulose and acrylic acid in the ratio of 1:8. Thereby, the combination formulation of 1:8 of hemicellulose and acrylic acid is considered to be an excellent candidate for further applications in near future.

CONCLUSIONS

Present research work shows that extracted hemicellulose was non-acetylated in nature as confirmed by FT-IR spectroscopy.

The non-acetylated hemicellulose was used for hydrogels preparation by reacting with acrylic acid in different (w/w) ratios. The maximum percentage of swelling (385%) was observed at neutral pH for that hydrogel formulation which has been prepared by the combination of hemicellulose and acrylic acid in the ratio of 1:8. Thereby, the formulation of 1:8 ratio of hemicellulose and acrylic acid is considered to be an excellent candidate for drug loading and drug release profile.

This non-acetylated hemicellulose-based hydrogel may also be useful in various applications including pH responsive drug delivery system, wound dressing, disposable diapers, scaffolds in tissue engineering etc in near future.

ACKNOWLEDGEMENT

The authors wish to acknowledge AKS University, Satna for providing necessary facility to conduct experiment feasible.

REFERENCES

1. Ahmed, E. M. (2013). Hydrogel: preparation, characterization, and applications. *Journal of advanced research*.
2. Hoare, T. R., & Kohane, D. S. (2008). Hydrogels in drug delivery: progress and challenges. Polymer, 49(8), 1993-2007.
3. Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J., & Thun, M. J. (2007). Cancer statistics, 2007. CA: a cancer journal for clinicians, 57(1), 43-66.
4. Kumar, S., & Negi, Y. S. (2012). Corn cob xylan-based nanoparticles: ester prodrug of 5-aminosalicylic acid for possible targeted delivery of drug. *J. Pharm. Sci. & Res*, *4*(12).
5. Kumar, S., & Negi, Y. S. (2014). Cellulose and xylan based prodrug of diclofenac sodium: synthesis, physicochemical characterization and in vitro release. *International Journal of Polymeric Materials and Polymeric Biomaterials*, *63*(6), 283-292.
6. Kumar, S., Negi, Y. S., & Upadhyaya, J. S. (2010). Studies on characterization of corn cob based nanoparticles. *Adv. Mater. Lett*, *1*(3), 246-253.
7. Singh, B. N. (2007). Modified-release solid formulations for colonic delivery. Recent patents on drug delivery & formulation, 1(1), 53-63.
8. Sinha, V. R., & Kumria, R. (2003). Microbially triggered drug delivery to the colon. European journal of pharmaceutical sciences, 18(1), 3-18.
9. World Health Organization: Health topics. http://www.who.int/topics/cancer/en (2009). Accessed 25 Feb 2009.