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A green and sustainable approach to utilize bio-ionic liquids for the selective precipitation of high purity agarose from an agarophyte extract†

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A few choline based bio-ionic liquids (bio-ILs) were employed for the first time for the selective precipitation of agarose from the hot seaweed extract of *Gracilaria dura* (an agarophyte) under ambient conditions. The method thus developed is much "greener" and economical in comparison with the methods widely practiced for agarose production. Among the bio-ILs, choline laurate was found to be the most effective for the isolation of agarose with a lower usage level (4.0%, w/w) with the yield of 14.0 ± 0.5% w/w. Agarose obtained by this process had the desired properties required for molecular biological applications and gel electrophoresis. Furthermore the bio-ILs were recycled and reused for subsequent batches of agarose isolation without compromising the yield and quality of biopolymers.

1. Introduction

Agarose is a hydrophilic linear galactan, composed of repeating disaccharide units, (1,3) linked β-D-galactopyranose (G) and (1,4) linked α-3,6-anhydro-L-galactopyranose (A) (Fig. 1a).[1] It forms weak to strong hydrogels depending on its concentration, which is ideal for diffusion and electro-kinetic movement of biopolymers such as DNA. The quality of agarose mainly depends on the quantity of sulphate present in it and is the basis of its commercial grading. Red seaweeds such as *Gelidium* spp., *Gracilaria* spp., *Acanthopeltis* spp., *Ceramium* spp., *Pterocladia* spp., and *Campylaephora* spp. are mainly used for the production of agar and agarose.[2] Native and cultivated *Gracilaria dura* (*G. dura*) of Indian seawater has been recently reported as a promising bio-resource for the preparation of agarose through alkali treatment and surfactant-induced selective precipitation processes.[3,4] In addition to these, there are a number of methods available in the literature describing isolation of agar such as repeated freeze–thaw, pressure syneresis, solvent precipitation *etc*.[5] Agarose (Fig. 1a), which is almost chargeless, is normally obtained by the purification of agar by chromatographic fractionations.[6]

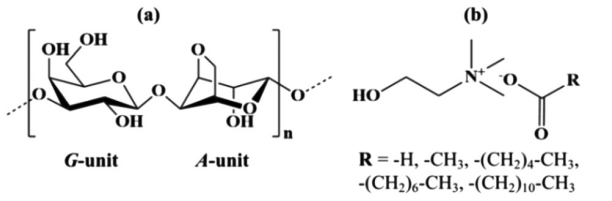

Fig. 1 Structure of (a) repeating unit of agarose and (b) choline based bio-ionic liquids used in this study.

Due to unique properties such as negligible vapour pressure, high boiling point, large electrochemical window, recyclability *etc*., ionic liquids (ILs) are much preferred choice for various applications such as in materials design,[7] to dissolve and extract various biopolymers[8] and as extraction media for polysaccharides.[9]

Although we have recently demonstrated the ability of few synthetic surfactants for the selective precipitation of agarose[4] a "greener" route for the isolation of the biopolymer was explored in the present work, where non-toxic and biodegradable ionic liquids of bio-origin (Fig. 1b) were employed for the job. Furthermore during the downstream processing, the bio-IL was successfully recovered from the spent solutions and was reused for the subsequent batches without compromising the purity and yield of agarose.

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