# Starch plasticization with choline dihydrogencitrate-based deep eutectic system

**Katarzyna Wilpiszewska**[*], **Dorota Skowrońska**

*West Pomeranian University of Technology, Szczecin, Faculty of Chemical Technology and Engineering, Department of Chemical Organic Technology and Polymeric Materials, ul. Pułaskiego 10, 70-322 Szczecin, Poland*
*[*]Corresponding author: e-mail: kwilpi@zut.edu.pl*

Two-component deep eutectic solvents (DES) based on choline dihydrogencitrate and glycerol or urea were tested as starch plasticizers. Thermal analysis techniques were applied to characterize the properties of starch/DES systems. The X-ray diffractometry measurements revealed a significant decrease in crystallinity indicating that used DES exhibited the ability to penetrate the ordered regions of potato starch, which is a necessary feature of a true plasticizer. However, the differential scanning calorimetry and dynamic thermal analysis results surprisingly showed an increase of $T_g$ of starch materials indicating chemical crosslinking at elevated temperature. The eutectic solvents based on choline dihydrogencitrate could act as a plasticizer and a simultaneously crosslinking agent.

**Keywords:** potato starch, deep eutectic solvents, starch plasticizing, choline dihydrogencitrate, thermal analysis.

## INTRODUCTION

Bearing in mind the environmental concerns looking for more environmentally friendly materials is necessary. Starch is one of the most abundant natural polymers. The native starch occurs in the form of semi-crystalline granules and their size and shapes depend on its botanical origin. Starch is widely used for food purposes. Its usage for industrial purposes requires converting it into a thermoplastic form, by introducing plasticizer at elevated temperature. Thermoplastic starch can be processed similarly to synthetic polymers. However, its added value is biodegradability of products and the biorenewable nature of raw material.

The plasticizer addition limits the brittleness of starch material. The most commonly used starch plasticizers are water multihydroxylic alcohols, e.g. glycerol. In recent years, a new type of plasticizing system – deep eutectic liquids (DES) has gained interest. They are a "green" alternative to ionic liquids and other harmful solvents[1]. The DES system can consist of two or more substances: hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). The characteristic feature is that its melting point is lower than the melting point of its components. For example, the most popular DES system based on choline chloride and urea is liquid at room temperature, although the melting points of the individual components are 302 °C and ~135 °C, respectively. It was stated that the low melting point of DESs can be related to the ability of the hydrogen bond acceptors to build a favorable network of hydrogen bond interactions with the other components of the system in such a way as to maximize the hydrogen bonds between all the different moieties[2]. The cation molecular symmetry has an additional effect. There are many reports on using DES systems for polysaccharides, however, they mostly refer to using DES for extraction of given polysaccharide from the biomass[3–4] or using DES as a reaction medium for biopolymer modification[5, 6]. Applying DES for starch plasticization is a promising approach, however, research on DES-starch interaction is still necessary.

An effective external plasticizer is a low molecular weight substance that interacts with a polymer, typically at elevated temperature (without a chemical reaction), exhibiting polymer dissolving or swelling ability. Theoretically, it would be therefore sufficient to select a plasticizer exhibiting a solubility coefficient value similar to the polymer's one. However, in the case of hydrophilic semicrystalline polymers (i.e. starch) ordered fractions are soluble only in the low-molecular substances able to form hydrogen bonds with polymer, and the hydrogen bond energy can be determined only for the simplest low-molecular-weight systems. Hence, it is necessary to use techniques that indirectly provide insight into the nature and intensity of plasticizer-polymer interactions.

Generally, the function of a plasticizer is to reduce interactions between macromolecules increasing the mobility of polymer chains. Experimentally, the degree of mobility of polymer chains can be assessed by determining the glass transition temperature using the DSC or DMA methods. Generally, shifting the Tg towards lower values is related to plasticizing efficiency. Due to the size dispersion of starch granules and the overlapping of signals from other transformations (gelation, crystallite melting), as well as the lower thermal conductivity of starch when compared to synthetic polymer – determination of the Tg value of starch is more complicated compared to synthetic polymers. Thus, using additionally the modulated DSC (MDSC), where reversible thermal transitions could be distinguished from the irreversible phase transitions, is recommended (because of the MDSC principle as well as starch gelatinization characteristics the thermal transition values are lower than those measured by conventional DSC[7]). MDSC should be used as a complementary method. Moreover, as the enthalpy and heat capacity changes could be associated with the thermal melting of crystallites, some evaluation of starch crystal structure could be probed by DSC. Other thermal analysis technique, i.e. DMA, besides Tg determination, allows additionally to evaluate the compatibility between the polymer and plasticizer. On the other hand, rheological studies allow to evaluate the penetration capacity of the plasticizer into the starch granules. The efficient plasticizer allows the gelatinization of native starch which indirectly indicates its solubility and corresponds to plasticization mechanism. In the

case of semi-crystalline polymers, such as starch, the effective plasticizer penetrates not only the amorphous fraction, but also between the ordered polymer chains, increasing the content of the amorphous fraction. Thus, the determination of the degree of crystallinity of the plasticized starch can also be used to evaluate the plasticization efficiency.

The goal of this paper was to investigate the effect of DES based on citric acid derivative, i.e. choline dihydrogencitrate (CCT) and glycerol (G) or urea (U) on starch plasticization. The physicochemical properties, i.e.: chemical structure (FTIR), viscosity (rheology), crystallinity (XRD), thermal (DSC, TG and DMTA methods) as well as mechanical properties of starch-based materials were characterized.

## EXPERIMENTAL

### Materials

Potato starch (moisture content ~18% wt.) was purchased from Zetpezet, Piła (Poland). Glycerol (G, technical pure) and urea (U, pure) were purchased from Chempur (Poland). Choline dihydrogencitrate salt (CCT) (≥98%) was a product of Sigma-Aldrich. In Fig. 1 the chemical structure of DES components was shown.
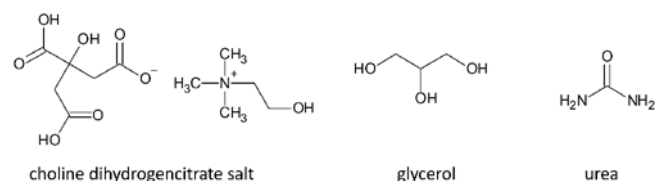


**Figure 1.** The chemical structure of DES components

### Preparation of deep eutectic solvents

Deep eutectic solvents CCT-G and CCT-U were prepared by stirring the components at 100 °C until the homogenous pellucid liquid was obtained (ca. 1 h).

### Preparation of starch/DES premixtures

Native potato starch and the proper amount of plasticizer (G, CCT_G, U and CCT_U) were ground in a mortar to obtain a paste. The systems contained 10–50 wt % of plasticizer depending on the tested parameter. The pastes were stored in a sealed container for 24 h before testing.

### Preparation of starch films

Premixtures were added into distilled water and stirred for 30 minutes at 90 °C. The obtained starch-based mixture was poured into Petri dishes (140 mm diameter) and dried in a climate chamber at 40 °C and RH 50% for 48 h. Starch films were stored in a climate chamber (25 °C, RH50%) for at least 24 h before testing.

### Methods

#### Fourier transform infrared spectroscopy (FTIR)

The FTIR analyses of the films were performed in Nexus FTIR Spectrometer Thermo Nicolet with Golden Gate ATR attachment. For each sample, 32 scans were taken from 4000 to 400 cm$^{-1}$. The resulting spectra were converted using the software OMNIC.

#### Rheology

The mixtures of starch with 10, 30 or 50% wt. DES before testing were stored in closed vials for 24 h at room temperature before testing. The rheological behavior was determined in air atmosphere, using rheometer Discovery-1 (TA Instruments) equipped with a parallel plate system. The temperature range was 50–220 °C, heating rate 10 °C/min, frequency 1 Hz. The complex viscosity as a function of temperature was obtained.

### X-ray diffractometry (XRD)

The crystallinity of starch/DES mixtures (before and after heating at 110 °C for 1 h) was analyzed by X-ray diffraction (X'pert Pro, PANalytical, operated at the CuK(alfa) wavelength 1,54 Å).

#### Differential scanning calorimetry (DSC)

Phase transitions of DES and starch/DES mixtures were investigated with the DSC technique (DSC Q100 TA Instruments) after 24 h storage. The aluminum hermetic pans (ca. 10 mg of the sample) with nitrogen as a cooling agent with a heating rate of 5 °C/min were applied. A standard run at the temperature range from 20 to 190 °C was performed.

#### Thermogravimetric analysis (TGA)

The thermal stability of starch/DES systems was investigated using TGA (Q500, TA Instruments). Tests were performed on platinum pans under 25 mL/min air form, in the temperature range of 40–700 °C at a heating rate of 10 °C/min.

#### Dynamic thermal mechanical analysis (DMTA)

Dynamic mechanical analysis (DMA Q800 TA Instruments) was used to measure tan δ of transition temperatures $T_\alpha$ and $T_\beta$ of starch/DES films. The measurements were carried out in a dual cantilever mode at a frequency of 1 Hz, heating rate of 3 °C/min, and a temperature range from –80 to 140 °C.

#### Mechanical properties

Mechanical properties were measured using a tensile tester (Instron 4026, Instron Corporation) equipped with a 1 kN load cell. The specimens (10 mm x 100 mm strips) were conditioned at RH = 50% for 24 h before tests. The initial grip separation and cross-head speed were 50 mm and 1 mm/min, respectively. The mechanical tensile data were averaged over ten specimens.

#### Wettability

The contact angle of starch films was measured using Dataphysics OCA 15EC optical contact angle analyzer. One drop of redistilled water (8 ml) was placed on the film surface and the contact angle was measured using DSA4 software. The initial contact angles in three replications were measured.

## RESULTS AND DISCUSSION

### Fourier transform infrared spectroscopy (FTIR)

In Fig. 2 A and B the FTIR spectra of native starch, DES: CCT_G or CCT_U and starch/DES 30% wt. systems (heated for 1 h at 110 °C), respectively were presented.
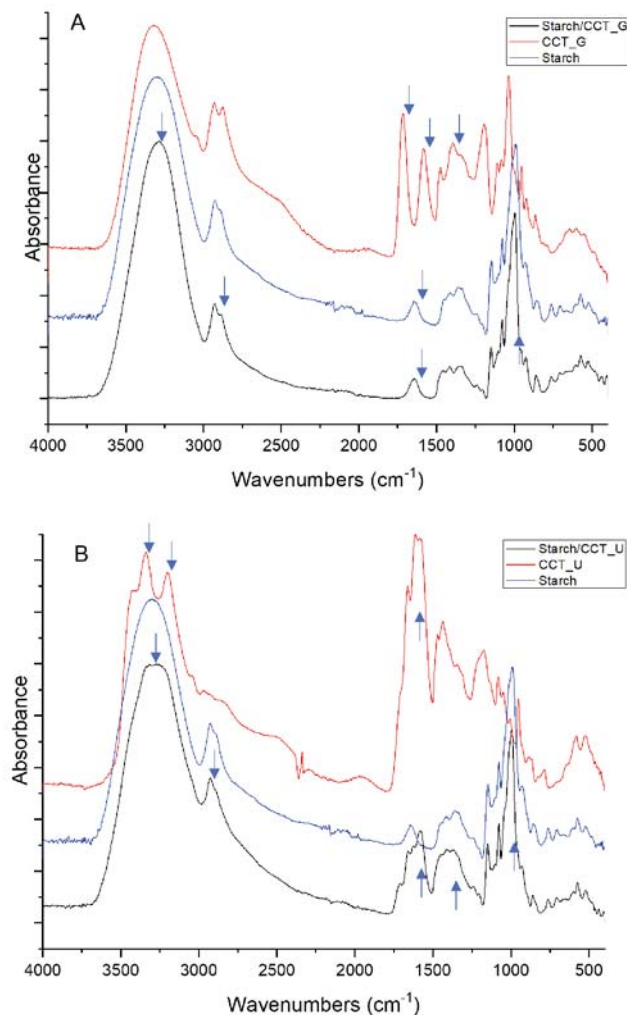


**Figure 2.** FTIR spectra of native starch, neat DES A) CCT_G and B) CCT_U and starch with 30% wt. DES

In the case of native starch the absorption band between 3600–3000 cm$^{-1}$ and at 2900 cm$^{-1}$ could be attributed to hydroxyl groups and to CH$_2$ stretching vibrations, respectively[8].

Protonated carboxylic groups (CCT) give a C=O band at about 1700 cm$^{-1}$ [9], and carboxylate (-COO-) gives a strong absorption band at about 1600 cm$^{-1}$, 1440 and 1325 cm$^{-1}$ [10]. For S/CCT_G the absorption band at 1715 cm$^{-1}$ and 1600 cm$^{-1}$ were noticed. Moreover, the overlapping of C-O starch band was observed (Fig. 1).

In the FTIR spectrum of S/CCT_U (Fig. 2 B) the characteristic urea absorption bands at 1600 cm$^{-1}$ attributed to carbonyl could be noticed. The NH group gives the signal at ca. 3200–3500 cm$^{-1}$, however this band overlapped with the OH band occurring in the same wavenumber values.

The special pattern, typical for native starch, in the region of 970 cm$^{-1}$ and 1200 cm$^{-1}$ was preserved in S/DES samples. Moreover, the band at ca. 1000 cm$^{-1}$ was slightly shifted toward a higher wavenumber (for starch 991.2 cm$^{-1}$, S/CCT_G 997.9 cm$^{-1}$, and S/CCT_U 993.2) indicating forming of new hydrogen bonds between starch chains and DES[11]. Additionally, the slight intensity increase of this signal (from 0.691 for starch up to 0.706 for S/CCT_U and 0.753 for S/CCT_G, respectively) indicated the amorphous state of starch[12]. It could suggest that both DES could penetrate between the starch semicrystalline arrays.

### Rheology

The viscosity measurements to some extent may indirectly provide insight into the nature and intensity of plasticizer-polymer interactions as well as the solvent power of the plasticizer for the polymer (for a plasticizer with a high degree of solvent power for the polymer, the polymer chains are stretched out, thereby offering strong resistance to flow resulting in a high dilute solution viscosity)[13]. The rheological behavior of starch systems, containing 10, 30 or 50% wt. DES, in a function of temperature, was evaluated – the curves were presented in Fig. 3A and B. Both systems exhibited a viscosity decrease (in most cases to ca. 1 Pa · s) at lower temperatures (up to 90 °C), which was probably the effect of melting DES. This phenomenon was observed also for starch systems with ionic liquid (1-allyl-3-methylimidazolium chloride) or eutectic choline chloride/succinic acid[14]. For the starch system with 10% wt. DES practically flat rheoviscometric curve was obtained indicating that no starch plasticization occurred. It might mean that the 10% wt. content of CCT-based DES was not enough for
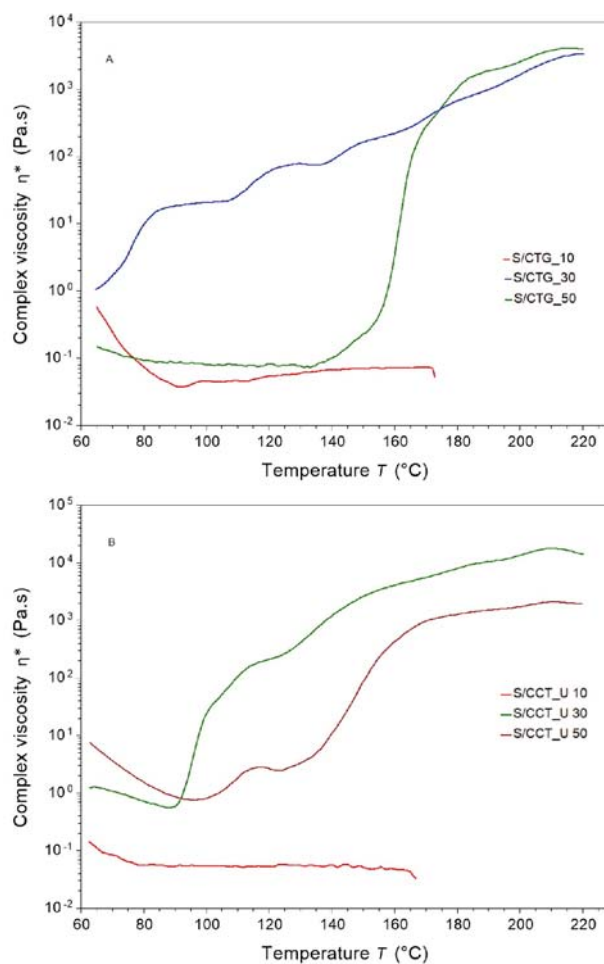


**Figure 3.** The rheometric curves of A) starch/CCT_G and B) starch/CCT_U systems containing 10, 30 or 50% wt.

efficient plasticization. Similar observations were noted for e.g. starch/urea systems, when the urea content did not exceed 10% wt. the hydrogen bonds between urea and polysaccharide caused the obstruction of the mobility of the starch segments leading to antiplasticization[15].

Interestingly, for the highest DES content the viscosity increase was sharp and intensive (from ca. 0.1 Pa·s up to 3970 and 2070 Pa · s for CCT_G and CCT_U, respectively). For the starch systems containing 30% wt. DES, after the initial drop, the viscosity increased gradually (up to ca. 3350 and 17700 Pa·s for the systems containing CCT_G and CCT_U, respectively). Zdanowicz et al[16] tested starch plasticization using molar ratio CCT to glycerol in a range from 1:2 up to 1:12, and noticed only a slight viscosity increase. However, DES content in the prepared starch mixtures was only 25% wt. The viscosity increase was probably the result of starch swelling and gelatinizing, additionally at the higher temperature the crosslinking reaction between polysaccharide and CCT could occur[17].

## X-ray diffractometry (XRD)

The effective plasticizer penetrates not only the amorphous fraction, but also between the ordered polymer chains, increasing the content of the amorphous fraction. The XRD method allows to determine the long-range crystalline order[18], and to indicate the plasticizer type (primary –penetrating only the amorphous region of polymer or secondary one – true plasticizer able to penetrate the ordered polymer structure). The XRD diffraction curves of starch/DES mixtures were presented in Fig. 4 A and B.

Native potato starch exhibits a crystal structure typical for tuber starch, i.e. type B, with characteristic signals at 6, ~15, 17, 22 and 24° [19]. Typically the degree of crystallization for potato starch is ca. 45%[19]. The XRD diffractograms of starch systems containing 30 wt.% and 50 wt.% DES after thermal treatment (after heating at 110 °C for 1 h) were presented in Fig. 4 A and B. Additionally, the starch mixtures with 30% wt. DES without thermal treatment were measured (stored in closed vials for 24 h at room temperature before testing).

For each thermally treated starch-based system the reflections intensity decreased with DES amount, thus the amorphous phase content increased. It means that the molecules of DES components had the ability to penetrate the order regions of starch. Such ability, i.e. swelling power, exhibit only secondary plasticizers[13]. However, some crystallinity remained, indicating incomplete granule melting. The samples for XRD were heated without the shear and friction forces induced by stirring, hot pressing or extruding[20]. Thus both, shear force and thermal treatment are required for starch granule disruption. The confirmation of that statement could be the fact that thermopressed (140 °C, 10 min, 12 tons) starch samples with CCT-G (molar ratio 1:6 up to 1:12)[16] were practically amorphous.

Interestingly, in XRD diffractograms of starch systems only mixed with CCT-G or CCT-U (no heating) the signals characteristic for $V_H$ type crystal structure at ca. 19° and 26° could be noticed[21]. This suggests that during starch plasticization the transformation of its crystalline structure occurred leading to helix-like inclu-
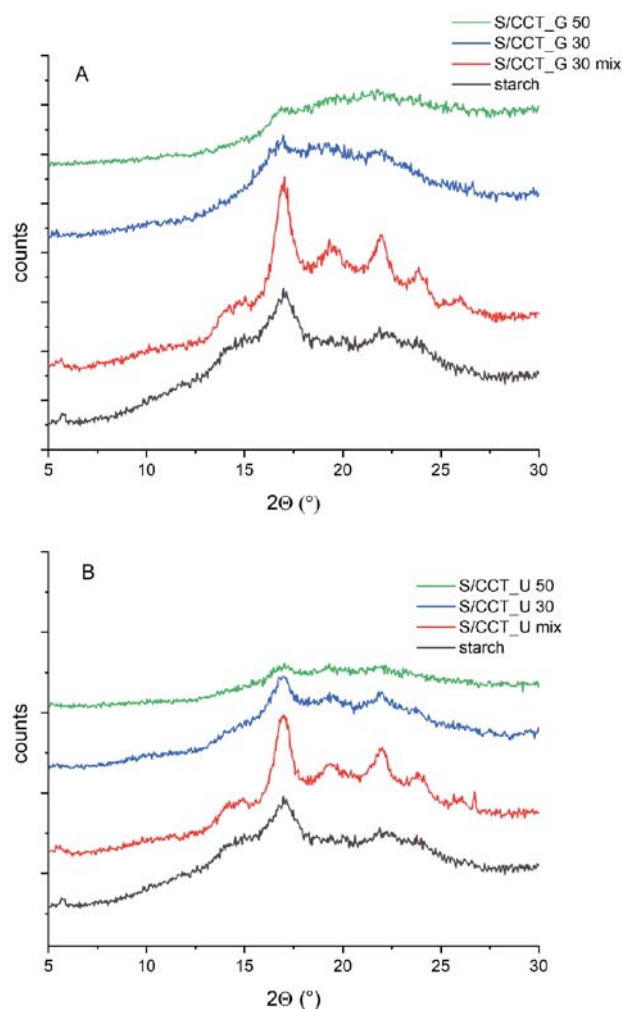


**Figure 4.** XRD diffractograms of native starch, starch/DES: A) starch/CCT_G 30, and B) starch/ CCT_U 30 mixtures, as well as starch/ DES systems after thermal treatment containing 30 and 50 wt.% DES, respectively

sion complexes. Generally, V type is a result of forming a single helix complex between amylose and alcohols or fatty acids. This type of structure was reported also for glycerol-plasticized starch[20]. However, as these signals were noted also for the system without glycerol, it could suggest that also citric acid component of DES exhibited the ability to complex forming.

## Differential scanning calorimetry (DSC)

DSC is the basic technique for evaluating thermal properties of polymers. Plasticization of polymer is assessed to increase mobility of macromolecules, and results in $T_g$ shift toward lower values in DSC thermogram[7]. For starch the measurement of $T_g$ could be difficult by DSC as the change in heat capacity is typically weaker than for the majority of polymers[20]. However, the effectiveness of the plasticizer could be evaluated by temperature and enthalpy of gelatinization, $T_{gel}$ and $\Delta H_{gel}$, respectively[22]. In Fig. 5 A and B the DSC thermograms of native potato starch, and starch-based systems were presented. Additionally, in Table 1 the thermal transitions temperature and enthalpy values were collected. The $T_{gel}$ decrease was noted: from 117 °C for unmodified starch to ca. 104 °C or 108 °C for starch plasticized with glycerol or urea, respectively. A similar phenomenom was reported for thermoplastic corn starch[20]. Additionally, for the S/U
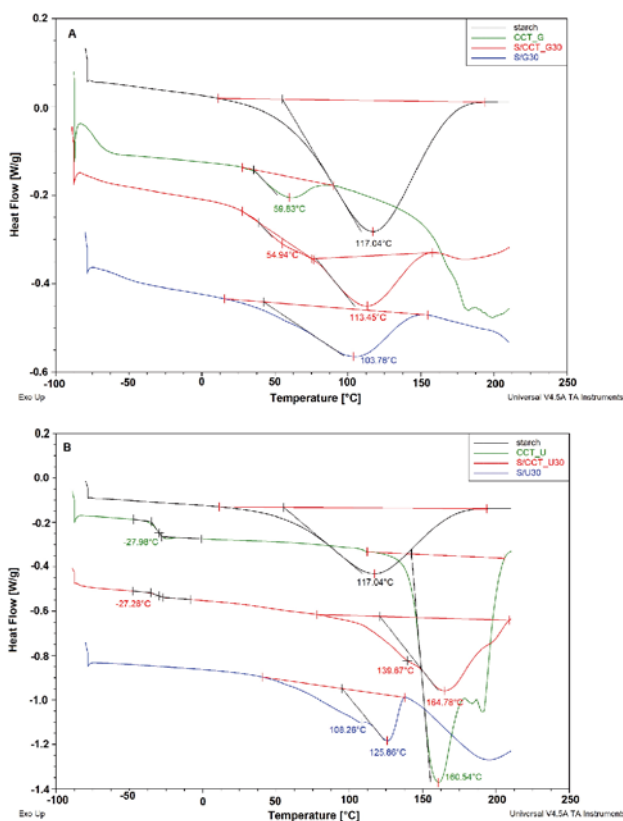
**Figure 5.** DSC thermograms of native starch, and starch-based systems: A) starch/G_30 and starch/CCT_G 30, and B) starch/U_30 and starch/ CCT_U 30

**Table 1.** The thermal properties (temperature $T_{gel}$ and enthalpy $\Delta H_{gel}$) of starch systems

| Sample | $T_{gel}$ [°C] | $\Delta H_{gel}$ [J/g] |
|---|---|---|
| Starch | 117.0 | 255 |
| S/G30 | 103.8 | 80 |
| S/CCT_G30 | 113.5 | 117 |
| S/U30 | 108.3 | 135 |
| S/CCT_U30 | 139.7 | 187 |

system the signal overlay could be noticed. It is known that when the urea content in starch-based mixture is ca. 30% wt. or higher the phase separation occurs indicating the excess of plasticizer[15]. The starch/CCTG_30 system exhibited slightly lower $T_{gel}$ value decrease (ca. 114 °C) when compared to S/G_30. Additionally, the second transition was noticed at ca. 55 °C, assigned to glycerol present in the system (observed also in the thermogram of neat glycerol).

The starch-based systems containing CCTU behaved differently, i.e. the $T_{gel}$ increased up to ca. 140 °C – Fig. 5B. Shifting of this signal was also reported for starch/CCTG systems where the DES components molar was ratio from 1:4 up to 1:12 (35 parts of DES per 100 parts of dry starch)[16], and it was assigned to the crosslinking reaction. Choline dihydrogencitrate was the component of both DES systems. It contains two carboxylic groups able to react with starch hydroxyls (three in each recurrent unit). Thus, at elevated temperature esterification could occur. Interestingly, for the starch/CCTU_30 system the signal assigned to the DES presence was noticed, i.e. ca. 165 °C, which could suggest that the phase rich in DES was formed. The $T_{gel}$ values correlate with the gelatinization enthalpies (calculated

from the areas under each endotherm or from the area under the deconvoluted peaks).

**Thermogravimetric analysis (TG)**

The thermogravimetric analysis allows to evaluate thermal stability of polymer materials. The TG thermograms of native potato starch and starch mixtures containing 30% wt. DES were presented in Fig. 6. For native starch at the temperature up to 100 °C water evaporation was noticed (the starch/DES mixtures were dried, thus this step did not occur). Native starch exhibited two-step weight loss. The highest one in a range of 150 up to 350 °C (with the maximum in DTG at 304 °C) was associated with dehydroxylation. The lower weight decrease in a range of 350–550 °C was assigned to thermal degradation[23]. Although the courses of weight loss curves for starch/DES systems were not as steep as this noted for starch, the natural polysaccharide exhibited higher thermal stability than its mixtures with DES. Moreover, it could be noticed that DES with glycerol component was more thermally stable than CCT-U (maximum in DTG thermograms: 231 °C and 205 °C, respectively). Similar observations were reported for starch with eutectic systems based on choline chloride containing glycerol or urea[24]. The complicated course of the starch/CCT_U DTG curve indicated a multi-stage transition process. Simeonov and Afonso[25] reported that urea could form carbonates with polyalcohols, even at moderate temperatures. Moreover, the reactions between carboxylic groups and urea[25] as well as starch hydroxyls were possible[24].
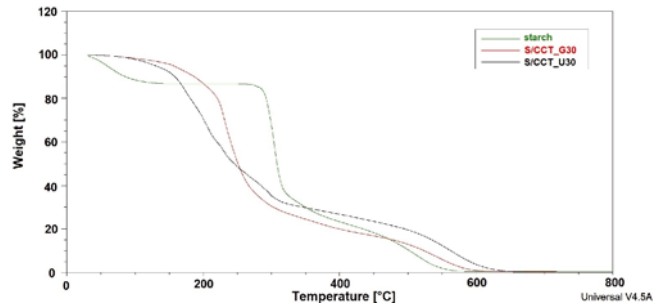


**Figure 6.** TG thermograms of native starch, and starch/DES mixtures

**Mechanical properties**

The tensile strength, Young's modulus and elongation at break of starch plasticized were presented in Fig. 7. The systems plasticized with neat glycerol and urea exhibited the lowest values of Young's modulus, as well as elongation at break, when compared to the DES-plasticized systems. The highest tensile strength ca. 3 MPa was noted for S/CCT_G30, slightly lower for S/CCT_U30. Moreover, their elongation at break was above glycerol and urea plasticized systems, and thus were less brittle. These improvements of mechanical properties were probably the result of crosslinking reaction between CCT and polysaccharide, and in consequence more dense structure of the starch system. It indirectly indicated that the presence of choline derivative was essential for the mechanical properties of starch films. Similar behavior, i.e. higher tensile strength with increase of crosslinking agent (citric acid) amount was reported also for other polysaccharide systems[26].
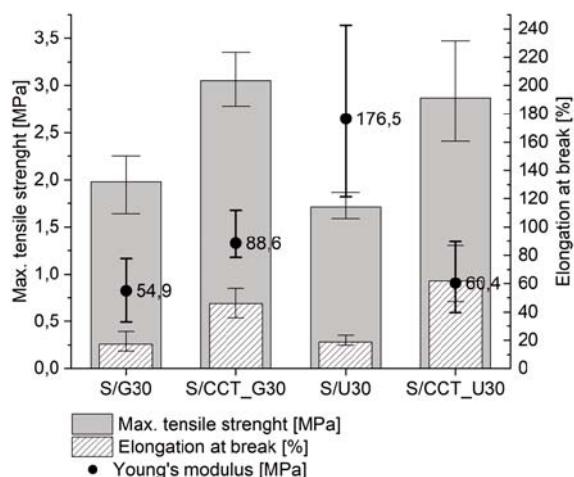
**Figure 7.** The tensile strength, Young's modulus and elongation at break of starch films

**Dynamic mechanical thermal analysis (DMTA)**

The evaluation of the loss factor (tan δ) as a function of temperature for S/DES films was shown in Fig. 8. The loss factor is sensitive to molecular motion and its peak relates to the glass transition temperature[13]. All the curves revealed two transitions indicating phase separation. For starch plasticized with glycerol it is known that the lower transition ($T_g$ ca. –50 ºC) is associated with glycerol rich phase, and the $T_\alpha$ value was ca. 90 ºC and indicating starch plasticization. For the starch/CCT_G system the glycerol-rich phase could still be observed, but the $T_\alpha$ was shifted toward higher values. i.e. ca. 130 ºC. The increase of the $T_g$ value directly indicates the crosslinking reaction between choline derivative and starch. The results correlate with DSC data. Similarly, for starch/CCT_U film the $T_\alpha$ was shifted up to ca. 125 ºC. Citric acid, which is a component of CCT is a known starch crosslinking agent, thus esterification via carboxymethyl bonds of CCT and starch hydroxyls could be expected[27].
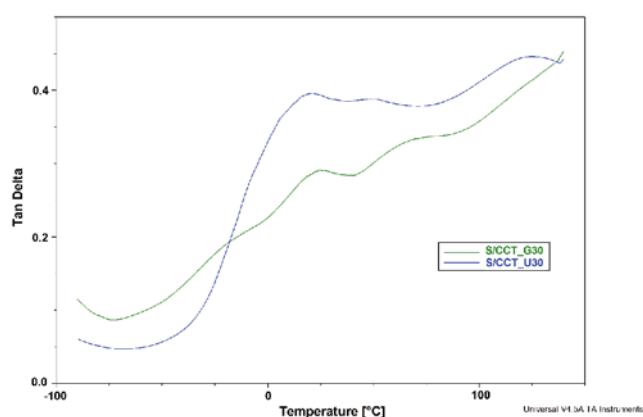


**Figure 8.** The DMTA curves of starch/DES systems

**Wettability**

In Table 2 the contact angle values of starch films plasticized with DES based on choline chloride and glycerol or urea were collected. For comparison, the films with neat glycerol and urea were given. By the contact angle measurements the attraction between the molecules in the water molecules and on polymer surface could be evaluated[28]. The stronger attraction the lower contact angle value could be noted. The lowest values of these

**Table 2.** Contact angles of starch films

| Sample | Average contact angle [°] |
| --- | --- |
| S/G30 | 17.0 ± 1.2 |
| S/CCT_G30 | 63.3 ± 2.2 |
| S/U30 | 13.5 ± 1.9 |

parameters were noted for the systems with glycerol and urea, respectively. Interestingly, higher increase of wettability was noted for the starch film plasticized with DES containing glycerol. The presence of choline dihydrogencitrate resulted higher contact angle. This probably was the result of esterification between CCT and OH groups of starch, which correlates with DSC measurement results.

**CONCLUSIONS**

Deep eutectic solvents based on CCT and glycerol or urea were tested as starch plasticizers. Thermal analysis techniques were applied to characterize the properties of starch/DES systems. The XRD measurements revealed, that used DES exhibited the ability to penetrate the crystalline regions of potato starch – acting as a secondary plasticizer. However, thermal treatment was necessary. Unexpectedly, the DSC and DMTA results showed an increase of $T_g$ of starch materials indicating chemical crosslinking at elevated temperature. Thus, CCT-based eutectic solvents could act as a plasticizer and a simultaneously crosslinking agent.

**FUNDING**

**LITERATURE CITED**

1. Sekharan, T.R., Chandira, R.M., Tamilvanan, S., Rajesh, S.C. & Venkateswarlu, B.S. (2022). Deep eutectic solvents as an alternate to other harmful solvents. *Biointerface Res. Appl. Chem*. 12, 847–860. DOI: 10.33263/BRIAC121.847860.

2. Migliorati, V. & D'Angelo, P. (2021). Deep eutectic solvents: A structural point of view on the role of the anion. *Chem. Phys. Lett.* 777. DOI: 10.1016/j.cplett.2021.138702.

3. Tan, Y.T., Chua, A.S.M. & Ngoh, G.C. (2020). Deep eutectic solvent for lignocellulosic biomass fractionation and the subsequent conversion to bio-based products – A review. *Biores.Technol*. 297. DOI: 0.1016/j.biortech.2019.122522.

4. Colombo Dugoni, G., Mezzetta, A., Guazzelli, L., Chiappe, C., Ferro, M & Mele, A. (2020). Purification of Kraft cellulose under mild conditions using choline acetate based deep eutectic solvents. *Green Chem.* 22, 8680–8691. DOI: 10.1039/D0GC03375H.

5. Hossain, M.A., Rahaman, M.S., Yelle, D., Shang, H, Sun, Z., Renneckar, S., Dong, J., Tulaphol, S. & Sathitsuksanoh. (2021). Effects of polyol-based deep eutectic solvents on the efficiency of rice straw enzymatic hydrolysis. *Ind. Crops Prod.* 167. DOI: 10.1016/j.indcrop.2021.113480.

6. Liu, Y. Xiaojie, Z., Shunhui, T., Lei, H., Xiaodong, Z. & Xiaoqing, Lin. (2021) Process optimization for deep eutectic solvent pretreatment and enzymatic hydrolysis of sugar cane bagasse for cellulosic ethanol fermentation. *Renew. Energy* 177, 259–267. DOI: 10.1016/j.renene.2021.05.131.

7. Xie, F., Liu, W.C., Liu, P., Wang, J., Halley, P.J. & Yu, L. (2010). Starch thermal transitions comparatively studied by

DSC and MTDSC. *Starch – Stärke* 62, 350–357 (2010). DOI: 10.1002/star.200900258.

8. Jiang, Q., Gao, W., Li, X., Liu, Z., Huang, L. & Xiao, P. (2011). Synthesis and properties of carboxymethyl Pueraria thomsonii Benth. starch. *Starch – Stärke* 63, 692–699. DOI: 10.1002/star.201100047.

9. Yanli, W., Wenyuan, G. & Xia, L. (2009). Carboxymethyl Chinese yam starch: synthesis, characterization, and influence of reaction parameters. *Carbohydr. Res.* 344, 1764–1769. DOI: 10.1016/j.carres.2009.06.014.

10. Spychaj, T., Wilpiszewska, K. & Zdanowicz, M. (2013). Medium and high substituted carboxymethyl starch: Synthesis, characterization and application. *Starch/Staerke* 65, 22–33. DOI: 10.1002/star.201200159.

11. Pushpadass, H.A., Marx, D.B. & Hanna, M.A. (2008). Effects of Extrusion Temperature and Plasticizers on the Physical and Functional Properties of Starch Films. *Starch – Stärke* 60, 527–538. DOI: 10.1002/star.201200159.

12. van Soest, J.J.G., Tournois, H., de Wit, D. & Vliegenthart, J.F.G. (1995). Short-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy. *Carbohydr. Res.* 279, 201–214. DOI: 10.1016/0008-6215(95)00270-7.

13. Immergut, E.H. & Mark, H.F. (1965). Principles of Plasticization. *Adv. Chem.* 48, 1–26. DOI: 10.1021/ba-1965-0048.ch001.

14. Zdanowicz, M. & Spychaj, T. (2011). Ionic liquids as starch plasticizers or solvents. *Polimery/Polymers* 56, 861–864. DOI: 10.14314/polimery.2011.861.

15. Zhang, Y. & Geng, X. (2020). Principle of biopolymer plasticization. Book chapter in *Processing and Development of Polysaccharide-Based Biopolymers for Packaging Applications* 1–19, Elsevier. DOI: 10.1016/B978-0-12-818795-1.00001-0.

16. Zdanowicz, M., Jędrzejewski, R. & Pilawka, R. (2019). Deep eutectic solvents as simultaneous plasticizing and cross-linking agents for starch. *Int. J. Biol. Macromol.* 129, 1040–1046. DOI: 10.1016/j.ijbiomac.2019.02.103.

17. Zdanowicz, M. & Johansson, C. (2016). Mechanical and barrier properties of starch-based films plasticized with two- or three component deep eutectic solvents. *Carbohydr. Polym.* 151, 103–112. DOI: 10.1016/j.carbpol.2016.05.061.

18. Warren, F.J., Gidley, M.J. & Flanagan, B.M. (2016). Infrared spectroscopy as a tool to characterise starch ordered structure – A joint FTIR-ATR, NMR, XRD and DSC study. *Carbohydr. Polym.* 139, 35–42. DOI: 10.1016/j.carbpol.2015.11.066.

19. Lopez-Rubio, A., Flanagan, B.M., Gilbert, E.P. & Gidley, M.J. (2008). A novel approach for calculating starch crystallinity and its correlation with double helix content: A combined XRD and NMR study. *Biopolymers* 89, 761–768. DOI: 10.1002/bip.21005.

20. Gamarano, D. de S., Pereira, I.M., da Silva, M.C., Mottin, A.C. & Ayres, E. (2020). Crystal structure transformations in extruded starch plasticized with glycerol and urea. *Polym. Bull.* 77, 4971–4992. DOI: 10.1007/s00289-019-02999-2.

21. Zobel, H.F. (1988). Starch Crystal Transformations and Their Industrial Importance. *Starch – Stärke* 40, 1–7. DOI: 10.1002/star.19880400102.

22. Zuo, Y., Gu, J., Tan, H. & Zhang, Y. (2015). Thermoplastic starch prepared with different plasticizers: Relation between degree of plasticization and properties. *J. Wuhan Univ. Technol. Mater. Sci. Ed.* 30, 423–428. DOI: 10.1007/s11595-015-1164-z.

23. Spychaj, T., Zdanowicz, M., Kujawa, J. & Schmidt, B. (2013). Carboxymethyl starch with high degree of substitution: synthesis, properties and application. *Polimery* 58, 503–511. DOI: 0.14314/polimery.2013.503.

24. Zdanowicz, M. (2020). Starch treatment with deep eutectic solvents, ionic liquids and glycerol. A comparative study. *Carbohydr. Polym.* 229, 115574. DOI: 10.1016/j.carbpol.2019.115574.

25. Simeonov, S.P. & Afonso, C.A.M. (2016). Basicity and stability of urea deep eutectic mixtures. *RSC Adv.* 6, 5485–5490. DOI: 10.1039/c5ra24558c.

26. Ghanbarzadeh, B., Almasi, H. & Entezami, A.A. (2011). mproving the barrier and mechanical properties of corn starch-based edible films: Effect of citric acid and carboxymethyl cellulose. *Ind. Crops Prod.* 33, 229–235. DOI: 10.1016/j.indcrop.2010.10.016.

27. Kapelko-Zeberska, M., Buksa, K., Szumny, A., Zieba, T. & Gryszkin, A. (2016). Analysis of molecular structure of starch citrate obtained by a well-stablished method. *LWT* 69. DOI: 10.1016/j.indcrop.2010.10.016.

28. Heydari A., Alemzadeh, I. & Vossoughi, M. (2013). Functional properties of biodegradable corn starch nanocomposites for food packaging applications. *Mat. Des.* 50, 954–961. DOI: 10.1016/j.matdes.2013.03.084.